Long-term outcome of repeated lead chelation therapy in progressive non-diabetic chronic kidney diseases

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Abstract

Background. Previous research suggest that repeated lead-chelation therapy decelerates progression of renal insufficiency in non-diabetic (non-DM) patients with high-normal body lead burden (BLB). Study findings are limited by relatively short-term follow-up and small sample size.

Methods. A total of 116 non-DM patients with chronic kidney diseases (serum creatinine level of 1.5–3.9 mg/dl), high-normal BLB (>60 μg and <600 μg) and no lead exposure history were randomly assigned to a chelation or control group in this 4-year clinical trial. For 3 months, the 58 chelation group patients received initial lead-chelation therapy with calcium disodium EDTA, and the 58 control group patients received placebos. During the ensuing 48 months, repeated chelation therapy was administered weekly to chelation group patients unless, on repeated testing, BLB was <60 μg; the control group patients received weekly placebo infusions for 5 weeks at 6-month intervals.

Results. Mean change in the glomerular filtration rate (GFR) in the chelation group was –1.8 ± 8.8 ml/min/1.73 m², as compared with –12.7 ± 8.4 ml/min/1.73 m² in the control group (P < 0.0001) at study end. Chelation group rates of decline in the GFR was lower than that in the control group, although they had similar decline rates before chelation. At study end, 18 patients, including 15 control group patients, had elevated serum creatinine levels to two times the baseline values. Both Cox and Kaplan–Meier analysis demonstrated repeated chelation therapy was the important determining factor of progression of renal insufficiency.

Conclusions. Repeated chelation therapies can, over a four-year period, slow progression of renal insufficiency in non-DM patients with high-normal BLB.

Keywords: body lead burden; glomerular filtration rate; long-term outcome; progression of renal insufficiency; repeated chelation therapy

Introduction

The renal toxic effects of lead are well established [1]. The most accurate techniques for measuring body lead burden (BLB) are bone X-ray fluorescence and calcium disodium EDTA (ethylene-diamine-tetra-acetate) mobilizations because blood lead levels (BLL) only reflect recent exposure to lead [2]. A person who has a BLB of <600 μg, as assessed by EDTA mobilization, is considered to have a normal BLB [2]. Clinical studies [3–8] utilizing EDTA-mobilization tests to determine BLB of chronic kidney disease (CKD) patients without known lead exposure indicate that long-term low-level environmental lead exposure was associated with renal insufficiency progression. A placebo-controlled, randomized, 2-year clinical trial [9] that repeated lead chelation therapy decreased progressive renal insufficiency in patients with CKD and high-normal BLB, even when related factors that influence levels were well controlled [10–15]. Additionally, none (0/32) of the chelation group patients had elevated serum creatinine concentrations ≥50% during treatment with repeated chelation therapy to maintain BLB <60 g, compared with 21.9% (7/32) of patients in the placebo group. Since a relatively small sample size and short duration of follow-up were noted limitations in the previous study, this 51-month placebo-controlled clinical trial assessed the long-term effect of repeated chelation in progression of renal insufficiency of 116 patients with high-normal BLB.

Methods

Subjects

The Medical Ethics Committee of Chang Gung Memorial Hospital (CGMH), Taipei, Taiwan, approved the study
protocol. All patients provided written informed consent. Patients aged 18–80 years with CKD were enrolled if they met the following criteria: serum creatinine concentrations between 1.5 mg/dl (132.6 μmol/l) and 3.9 mg/dl (344.8 μmol/l); had follow-up at CGMH for >1 year with a decrease in estimated glomerular filtration rate (GFR) of <5 ml/min over a period of at minimum 6 months; no history of exposure to lead or other heavy metals; no history of receiving lead chelation therapy; and, a high-normal BLB (60–600 μg, as measured by EDTA mobilization testing and 72-h urine collection). Specific renal diagnoses were based on the patient history, laboratory results, renal imaging and renal histological examination [16]. The exclusion criteria were as follows: renal insufficiency with a potentially reversible cause, or drug-induced nephrotoxic effects; systemic disease-related nephropathy such as diabetic nephropathy, lupus nephritis; use of steroid or immunosuppressive drugs; rapidly progressive glomerulonephritis or an elevated 24-h urinary protein excretion (>8 g/day). Blood pressure was controlled with diuretics and angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor–antagonists (ARA), with or without non-dihydropyridine calcium-blocking agents. Patients with normal blood pressure were not administered ACEI or ARA. Patients with poorly controlled blood pressure (>160/95 mmHg), hyperlipidaemia (>260 mg/dl), protein intake exceeding 1.2 g/kg/day for >3 months were excluded during the sample collection period. No patient received vitamin D3 supplements or erythropoietin treatment. A nutritionist reviewed each patient’s dietary intake at 3–6 month intervals, and 24-h urea excretion was measured every 3 months to determine nitrogen balance and dietary compliance [17]. BLL and BLB were measured as described previously [3,4,9]. BLB was determined utilizing EDTA-mobilization test methods developed by Emmerson and modified by Behringer et al.

**Study protocol**

**Data-collection period.** Baseline BLL, haemoglobin levels and BLB in 250 patients were determined during this 12-month period prior to the study. Serum creatinine, blood urea nitrogen and cholesterol, as well as urinary protein, creatinine and urea excretion, were measured at 3-month intervals with an auto-analyser system (Hitachi, Japan) to ensure that patients met enrolment criteria. Urinary excretion measurements were the average of two consecutive 24-h urine collections. Renal function was analysed by measuring creatinine clearance (Ccr) and estimated GFR (both in ml/min/1.73 m²) [19].

**Intervention period.** The data-collection period was followed by a single-blind, randomized, placebo-controlled study, lasting 51 months, in which all patients were randomly assigned to receive a placebo or chelation therapy during the first 3 months and then received either placebo or repeated chelation therapy, when necessary, over the following 48 months. Laboratory measurements were performed at 3-month intervals during this period. In total, 116 patients met enrolment criteria and were randomly assigned to the control (n = 58) or chelation group (n = 58). During the first 3 months, the chelation group patients received intravenous infusions of one vial (1 g) of calcium disodium EDTA mixed with 200 ml of normal saline over a 2-h period weekly unless BLB fell to <60 μg (0.29 μmol). Control patients were administered weekly infusions of one vial (20 ml) of 50% glucose mixed with 200 ml normal saline over 2 h for 5 weeks [9]. The chelation group patients received repeated lead chelation therapy, as in the first 3 months, if their serum creatinine levels increased above pre-chelation basal levels and BLB exceeded 60 μg, or if their BLB was >60 μg at regular 6-month BLB reassessments during the intervention period. Control group patients received a placebo weekly for 5 weeks every 6 months during this period.

**Outcome measures**

The primary endpoint was a temporal change in Ccr or estimated GFR during the intervention period. A secondary endpoint was serum creatinine level increased to twice times that at baseline or need for dialysis.

**Statistical analysis**

The sample size was calculated with PASS software (power analysis and sample-size package, NCSS statistical software). For a two-sided test at the 0.05 significance level, a sample size of 118 patients (each group, n = 58) is adequate for detecting difference between treatment groups at 3-month intervals for rate of change to the GFR of 0.31 ml/min [3]. The sample size estimation had a power of 0.95. The Cox proportional-hazards model was employed to determine the possible variables [3–8] to predict the secondary endpoint. Differences in rates of progressive CKD between the two groups were analysed using the Chi-square test, Student’s t-test and Mann–Whitney U-test. The cumulative percentage of all patients with the secondary endpoint was measured with the Kaplan–Meier method and log rank test. All P-values were two-tailed and <0.05 were significant, and all results are presented as means ± SD. An intention-to-treat analysis was performed. Additionally, a sensitivity analysis was performed that assigned mean values for the chelation group to controls lost during follow-up and assigned mean values for the control to chelation patients lost during follow-up.

**Results**

**Baseline data-collection period**

In total, 116 met enrolment criteria and entered into the interventional period (Figure 1). The yearly rate of reduction in GFR for the chelation group was 2.3 ± 7.0 ml/min/1.73 m², which was similar to that of the control group (2.5 ± 4.5 ml/min/1.73 m², P = 0.8030 by Mann–Whitney U-tests) prior to the clinical trial.

**Intervention period**

**Initial chelation therapy.** Both groups had similar baseline characteristics (Table 1). After 3 months of lead chelation therapy, the BLB of chelation group patients decreased to 32.2 ± 16.9 μg (range, 9.2–58.0 μg), and their BLB decreased to 3.9 ± 1.3 μg/dl (range, 1.9–7.1 μg/dl). The average therapeutic dose of
Calcium disodium EDTA was 4.8 ± 2.0 g (range, 4–12 g). After initial chelation therapy, improvement in renal function of the chelation group was noted (Table 2).

Repeated chelation therapy. Throughout the intervention period, the two groups did not differ in body mass index, mean arterial pressure, serum cholesterol level, daily urinary protein excretion and daily protein

(a) Inclusion criteria for patients with serum creatinine ≥1.5 and ≤3.9 mg/dl, without previous lead exposure, and without diabetes mellitus

(b) Exclusion criteria for patients with body lead burden ≥60 µg or >600 µg and serum creatinine >4.0 mg/dl

(c) Exclusion criteria for patients with body lead burden ≥60 µg and <600 µg and serum creatinine >4.0 mg/dl

(d) Exclusion criteria for patients with poor blood-pressure control, poor cholesterol and protein intake control, drug-related acute renal failure, rapid progressive renal insufficiency, severe daily proteinuria

Fig. 1. Flow chart showing the enrolment and status of patients.
Table 1. Baseline characteristics of study patients with high normal body lead burden (60 µg and <600 µg) at the entry of clinical trial

<table>
<thead>
<tr>
<th>Variables</th>
<th>Chelation group (n = 58)</th>
<th>Control group (n = 58)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Y/O)</td>
<td>57.8 ± 10.9 (33–79)</td>
<td>57.3 ± 13.2 (25–80)</td>
<td>0.8006</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>47/11</td>
<td>42/16</td>
<td>0.3798a</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.2 ± 2.9 (20.4–33.1)</td>
<td>25.2 ± 3.5 (17.7–33.7)</td>
<td>0.9552</td>
</tr>
<tr>
<td>Smoking</td>
<td>4</td>
<td>6</td>
<td>0.7406</td>
</tr>
<tr>
<td>Serum Cr (mg/dl)</td>
<td>2.4 ± 0.8 (1.5–4.0)</td>
<td>2.4 ± 0.7 (1.5–4.0)</td>
<td>0.9590</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73 m²)</td>
<td>41.9 ± 13.8 (12.8–72.4)</td>
<td>40.2 ± 9.5 (17.2–65.6)</td>
<td>0.4425</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min/1.73 m²)</td>
<td>36.8 ± 12.7 (16.5–60.5)</td>
<td>36.0 ± 11.2 (18.0–62.0)</td>
<td>0.6925</td>
</tr>
<tr>
<td>Blood lead levels (µg/dl)</td>
<td>5.0 ± 2.2 (1.1–12.3)</td>
<td>5.1 ± 2.6 (1.3–14.8)</td>
<td>0.8305</td>
</tr>
<tr>
<td>Body lead burden (µg)</td>
<td>164.1 ± 111.1 (60.0–574.0)</td>
<td>151.5 ± 92.6 (63.0–530.0)</td>
<td>0.4908</td>
</tr>
<tr>
<td>Daily proteinuria (µg/day)</td>
<td>0.82 ± 0.92 (0.03–3.7)</td>
<td>0.89 ± 0.91 (0.03–4.5)</td>
<td>0.2996a</td>
</tr>
<tr>
<td>Daily protein intake (g/kg)</td>
<td>0.95 ± 0.20 (0.5–2.0)</td>
<td>0.93 ± 0.20 (0.5–1.8)</td>
<td>0.4518</td>
</tr>
<tr>
<td>Hypertension</td>
<td>43</td>
<td>45</td>
<td>0.8285a</td>
</tr>
<tr>
<td>Using CEI or ARA</td>
<td>40</td>
<td>41</td>
<td>0.9999a</td>
</tr>
<tr>
<td>Using dihydropyridine CCB</td>
<td>10</td>
<td>6</td>
<td>0.4213a</td>
</tr>
<tr>
<td>Hyperlipidaemia underlyng renal disease</td>
<td>19</td>
<td>12</td>
<td>0.2077a</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>33</td>
<td>35</td>
<td>0.8505a</td>
</tr>
<tr>
<td>Chronic interstitial nephritis</td>
<td>9</td>
<td>6</td>
<td>0.5813a</td>
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<tr>
<td>Hypertensive nephropathy</td>
<td>5</td>
<td>5</td>
<td>0.9999a</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>3</td>
<td>4</td>
<td>0.9999a</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>8</td>
<td>0.9999a</td>
</tr>
</tbody>
</table>

Hyperlipidaemia: serum cholesterol >240 mg/dl after diet control; hypertension: blood pressure >140/90 mmHg at least twice measurements and with anti-hypertensive drugs. CEI: converting enzyme inhibitors. ARA: angiotensin II receptor antagonists, CCB: calcium channel blockers.

*aData were measured by the Chi-square with Fisher test.

*bData by Mann–Whitney U-method. Other data were measured by the Student’s t-test.

During this period, 10 patients in the chelation group required one course, 21 required two courses, 22 required three courses, and four needed four courses of repeated chelation therapy. However, renal function improved after BLB was again reduced by repeated chelation therapy. The mean dose of calcium disodium EDTA for repeated chelation therapy was 8.2 ± 3.7 g (range, 3–17 g). At study end, BLL (3.5 ± 1.5 µg) and BLB (32.2 ± 16.9 µg) for the chelation group patients were lower than those (BLL: 6.0 ± 4.1 µg, P = 0.0306) (BLB: 160.5 ± 108.2 µg, P < 0.0001) for the control group. Improved renal function (GFR) persisted for 24 months in the chelation group as chelation therapy was repeated after the initial course of therapy. However, at study end, GFR in chelation group patients (35.4 ± 17.0 ml/min/1.73 m²) was superior to that of the control group (23.7 ± 10.8 ml/min/1.73 m²; P < 0.0001) (Table 3). The yearly rate of decrease in GFR during this period for the chelation group was 1.2 ± 2.3 ml/min/1.73 m² and less than that for the control group (2.5 ± 2.0 ml/min/1.73 m², P = 0.0017) (Table 2) (Figure 2). Three patients in each group did not complete the final 48-month intervention period with no effect to experimental results, as determined by a sensitivity analysis (Table 4). No side effects of repeated lead chelation therapy were observed during the 4-year study period. Eighteen patients reached the secondary endpoint during the study period, including 15 control group patients and three chelation group patients (log rank test, P = 0.0019) (Figure 3). Among these 18 patients, one in the chelation group and two in the control group received renal replacement therapy.

Cox regression multivariate analysis revealed that age, baseline serum creatinine, BLL, daily urine protein excretion and no repeated lead chelation therapy were significant risk factors for progression of renal insufficiency, even after adjusting for other related factors (Table 5).

Discussion

Experimental results for this 4-year study indicate that repeated chelation therapy can, over the long-term, slow progression of renal insufficiency in non-diabetic patients with high-normal BLL; these results are consistent with those obtained by a 2-year chelation therapy study [9] and animal study [20]. However, the definition of endpoint to doubling of serum creatinine in the current study was different from a 1.5-fold increase in serum creatinine in the previous article. Moreover, a longer follow-up (51 months vs 27 months) and larger sample size (116 subjects vs 64 subjects) with higher power of pre-study assessment make this investigation draw a more definite conclusion than a previous trial about repeated chelation therapy in treating progressive renal insufficiency in CKD patients. Moreover, some findings of this study are different from those of a previous trial [9]. First, at the end of month 27, no significant difference exists for rate of progressive renal insufficiency between the two study groups and significant differences presented at the end of month 39. This finding conflicts with that of a previous 27-month clinical trial [9] and may be explained by different study subjects. Second, at study
end, the chelation and control groups differed in terms of the GFR by \( \sim 10.9 \) ml/min. This finding indicates that treated patients may delay dialysis therapy by roughly 4 years, given the rate of decline in the GFR of \( \sim 2.5 \) ml/min/year in the control group. Comparing with a previous 2-year clinical trial delaying renal replacement therapies by about 2 years [9], it seems that longer duration of repeated chelation therapy may cause longer postponement of renal replacement therapies. Third, at study end, 18 patients, 15 control group patients and three chelation group patients reached the secondary endpoint. Cox multivariate analysis demonstrated that repeated chelation therapy significantly affects the progression of renal insufficiency. An animal study [21] proved that EDTA can rapidly reduce brain and kidney lead contents; however, bone lead levels respond poorly to the
chelating agent and, therefore, the skeleton becomes a permanent source of poisoning. Hence, repeated chelation therapy is needed to maintain low-level lead content in the kidney. These novel findings further verify the hypotheses that repeated chelation therapy effectively retards CKD progression in patients with high-normal BLB.

Whether elevated BLB is a cause or consequence of CKD has received considerable debate [22]. However, most studies indicate that BLB causes, rather than results from, decreased renal function [23,24]. Chemical and histological studies of trans-iliac biopsies from 153 dialysis patients [25] proved that chronic renal failure does not cause lead accumulation in bone.

The mechanism by which lead chelation therapy improves renal function and slows progression of renal insufficiency requires elucidation. It is known that EDTA chelation can reduce serum levels of other divalent ions [20] such as calcium, magnesium, zinc, cadmium or iron. Studies [26,27] of cadmium plant employees or those residing in cadmium-polluted areas have demonstrated that cadmium is a nephro-toxic agent. However, cadmium’s toxic effect remains unclear in the general population. Additionally, patients with a history of heavy metal exposure...
were excluded in this study. Environmental low-level exposure to lead can increase activation of the renin-angiotensin-aldosterone system in animals and humans [20,28]. Lead chelation therapy combined with removal of copper and zinc, which are co-enzymes of the angiotensin II converting enzyme, likely inhibits renin and angiotensin II activity. However, no significant reduction of proteinuria in chelation group patients occurred after chelation therapy. Although animal studies [29,30] suggest that iron may play a role in progressive renal failure, there is no potential role of the same findings in humans [29,30]. Moreover, few studies observed that exposure to environmental low-level EDTA chelatable ions other than lead affect renal function. Conversely, chronic low-level—not high-level—lead exposure can increase the concentration of reactive oxygen species and nitric oxide inactivation [31,32]. Lead chelation therapy reduces the levels of reactive oxygen species in tissues and restores nitric oxide activation [31,32], likely improving renal function and slowing the renal insufficiency progression in the chelation group patients. Hence, it is reasonable to propose that removal of body lead—and not other ions—retnads progressive chronic nephropathy.

Although using Cr and the estimated GFR indicators to determine changes in renal function limits the results of this study [33], estimated GFR by Levey et al. [19] demonstrated a strong association with isotopic GFR ($r^2 = 0.91$). Other limitation of this study is its relatively small sample size. However, pre-study sample size estimation had adequate power ($>0.8$) for a clinical trial. Furthermore, the reasons why nine patients did not complete the study may have biased experimental results. A conservative sensitivity analysis was performed after the study and did not alter experimental results. However, the study indeed suffers from a major bias, namely the fact that the primary endpoint was in itself an indication to repeated chelation therapy in the chelation group, whereas the control group received placebo injections only given at a few and predetermined time points. The bias may limit the generalizability of our findings to non-diabetic patients with CKD.

In conclusion, this clinical study demonstrates that the repeated chelation therapy can, over a four-year period, slow progression of non-diabetic patients with CKD and high-normal BLB. Additionally, no side effects were noted for repeated lead chelation therapy in this 51-month clinical trial and other studies [34]. These investigational findings indicate that repeated lead chelation therapy may be an effective and safe means of treating CKD. Further study is required to confirm these findings.

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**Conflict of interest statement.** None declared.

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