Published in final edited form as:

J Pediatr. 2011 March ; 158(3): 480–485.e1. doi:10.1016/j.jpeds.2010.08.036.

Efficacy of succimer chelation of mercury at background exposures in toddlers: a randomized trial

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Abstract

Objective—To examine whether succimer, a mercaptan compound known to reduce blood lead concentration in children, reduces blood mercury concentration.

Study Design—We used samples from a randomized clinical trial of succimer chelation for lead-exposed children. We measured mercury in pre-treatment samples from 767 children. We also measured mercury in blood samples drawn 1 week after treatment began (N=768) and in a 20% random sample of the children who received the maximum 3 courses of treatment (N=67). A bootstrap-based isotonic regression method was used to compare the trend over time in the difference between the adjusted mean mercury concentrations in the succimer group and the placebo group.

Results—The adjusted mean organic mercury concentration in the succimer group relative to the placebo group fell from 99% at baseline to 82% after three courses of treatment (p for trend = 0.048), but this resulted from the prevention of the age-related increase in the succimer group.

Conclusion—Succimer chelation for low level organic mercury exposure in children has limited efficacy.

Keywords

Heavy metals; thimerosal; methyl mercury; amalgam

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The authors have indicated they have no financial relationship relevant to this article to disclose.

INTRODUCTION

Children can be exposed to metallic mercury and its vapor from a variety of sources, including broken mercury vapor lamps and thermometers, contaminated work clothes and tools, and dental amalgam fillings. Methyl mercury is a common food contaminant, especially of fish.[1] High exposures to elemental or inorganic mercury produce acrodynia, with proteinuria, mood and visual changes, and a rash.[2] High prenatal exposure to methyl mercury produces a cerebral palsy-like illness in children; there have been three major outbreaks, two in Japan from contaminated fish,[3, 4] and one in Iraq from consumption of seed wheat preserved with methyl mercury.[5] Methyl mercury is now universally regarded as toxic for the fetus, and there are recommended limits for consumption of contaminated fish by women of reproductive age.[6, 7] Because the aforementioned mass poisoning episodes demonstrated methyl mercury's neurodevelopmental toxicity, two large prospective studies of children with relatively high prenatal exposures have been in progress, one in the Seychelle Islands,[8] off the east coast of Africa, and the other in the Faroe Islands, north of Scotland.[9] In the Faroes, where the methyl mercury exposure comes from consumption of both contaminated fish and pilot whale meat, children with greater measured prenatal exposures showed lower scores on tests of neuromotor coordination, language, and executive functions at 7 years of age. At 14 years of age these associations were diminished in number and strength.[10] In the Seychelles, where methyl mercury exposure comes from a diet high in marine fish, no consistent pattern of associations was found between prenatal methyl mercury exposure and neurodevelopmental test scores through age 9 years.[11, 12] The other well known form of organic mercury is ethyl mercury, present in thimerosal, a vaccine preservative and, as merthiolate, a common topical disinfectant. Thimerosal was removed from all US vaccines except influenza beginning in 1999; however, suspicion arose that thimerosal produced brain damage resulting in autism or autism like disorders in children who received the older vaccines. There is now scientific consensus, as stated in a 2004 report from the US Institute of Medicine, that "the body of epidemiological evidence favors rejection of a causal relationship between thimerosal-containing vaccines and autism." [13]. Nonetheless, in a national survey conducted in 2008, "...24 percent [of adults] said that because vaccines may cause autism it was safer not to have children vaccinated at all. Another 19 percent were not sure." [14]

Although chelating drugs can remove mercury from the body and prevent further deterioration in acute situations,[15] they have not been shown to reverse damage to the central nervous system or improve neuropsychological functions.[16–18] In experimental studies, succimer (meso-2,3-dimercapto-succinic acid, DMSA), an orally administered chelating drug labeled for pediatric lead poisoning [19], does not remove methyl mercury from the brain of poisoned rodents[20]. Succimer does increase the urinary excretion of methyl mercury in rodents[21] and in persons who consume presumably contaminated sport fish [22]. This is perhaps because methyl mercury in water or serum is methyl mercuric cation, and the ionized metallic moiety is capable of interacting with the sulfurs from the succimer molecule. Dimethyl mercury, the fully organified (and extremely toxic) form, would not be expected to be chelatable at all.

Some parents of children with autism have sought chelation therapy to lower their child's body burden of mercury in the hope of reducing some of the symptoms of autism. Anecdotally, succimer is used for this, although this practice is based on only one small uncontrolled study (which used other agents also)[23] that has not been accepted as evidence of safety or efficacy [24]. The US National Institute of Mental Health proposed a trial of succimer in children with autism spectrum disorders, but it was halted before enrollment began when a new laboratory study provoked safety concerns, and evidence for direct

benefit to study participants was found to be lacking.[25] Presently, for acute mercury poisoning, succimer or D-penicillamine are used in the US; unithiol, which is the sodium salt of dimercaptopropane sulfonic acid (DMPS) and Nacetyl-D-penicillamine are available outside the US. [15] However, no agent is labeled for treating mercury exposure in children, and there are few data to provide guidance for the use of chelation to reduce mercury in young children.

We have completed a randomized clinical trial of succimer for lead poisoning in 780 children aged 12–33 months, called the Treatment of Lead-exposed Children trial, or TLC[26]. In that study, we observed the largest estimated mean difference between groups in blood lead concentrations, approximately 11 $\mu\text{g/dL}$ ($0.53 \mu\text{mol/L}$), at one week after the beginning of treatment. During the 6 months after initiation of treatment, the mean blood lead concentration of the succimer-treated children was lower than that of placebo-treated children.[27] In that trial, succimer did not produce any change in IQ or behavior in children, even though it acutely lowered their blood lead concentrations.[28] The samples remaining from the TLC trial have allowed us to study the effect of succimer in reducing blood mercury in toddlers, and, thus, to fill a gap in the scientific literature that is unlikely to be addressed any other way. Using the blood samples from the TLC trial, we measured blood mercury concentration at one week before randomization and treatment; at one week after treatment initiation, which was the point of maximum efficacy of succimer for reducing blood lead concentration; and after three courses of treatment, when the maximum amount of drug had been administered. The aim of the present study is to determine the efficacy of succimer in reducing blood mercury in toddlers after one week of treatment with succimer and after three courses of treatment with succimer.

METHODS

The blood samples and data come from the TLC study, a 4-site, placebo-controlled randomized trial. The study was conducted between September 1994 and June 2003. It accepted referral of children who were 12 to 33 months of age, and had blood lead concentrations between 20 and 44 $\mu\text{g/dL}$. Children who had confirmed venous blood lead concentrations between 20 and 44 $\mu\text{g/dL}$ and lived in cleanable housing (by vacuuming, damp mopping, or wiping to minimize lead exposure) had a second screening visit about one week later.[26] If the blood lead concentration at the second visit was also between 20 and 44 $\mu\text{g/dL}$, the children entered the randomization phase, and their house was cleaned between their second blood lead measurement and the beginning of treatment. TLC enrolled 780 children; parents or guardians signed informed consent documents covering 3 phases of the study, including all activities leading up to randomization, and for later follow-up. For the current report, we constructed a data set that included demographics, treatment information and mercury levels but not personal identifiers. We applied for and received human subjects research exemption for this analysis from the Office of Protection from Research Risks at the NIH.

Treatment assignments were randomized within strata of the four clinical centers, six categories of body surface area, two strata of blood lead concentrations ($\leq 25 \mu\text{g/dL}$, or $> 25 \mu\text{g/dL}$) and languages (English or Spanish).[26] Children could receive up to 3 courses of succimer or placebo. McNeil Consumer Products (Fort Washington, PA, U.S.A.) provided unmarked succimer (Chemet 100 mg) and placebo capsules of identical appearance. The dose was calculated on a body surface area basis.[26] The courses of treatment were 26 days long, with the first 7 days at a higher loading dose. Children were scheduled to return for clinic visits at 7, 28 and 42 days after the beginning of each treatment course. When a child who was receiving succimer had a blood lead concentration of $\geq 15 \mu\text{g/dL}$ at the 6- and 8-week follow-up visit of the first or second course, an additional course of

treatment was initiated. Children given placebo were assigned to retreatment to match the frequency of retreatment of children given succimer within the blocks used in the initial randomization.[26]

Measurement of Mercury

For the current study, the outcomes were total mercury and organic mercury concentrations in the blood samples. The Division of Laboratory Sciences at the National Center for Environmental Health at CDC analyzed all blood samples drawn about one week before randomization (baseline) and one week after treatment began. Of 338 children who finished 3 courses of treatment, we drew a 20% random sample to test the mercury concentrations at the completion of all three courses. The identification numbers of samples were recoded by the data coordinating center to delink the association between the child's clinical record at the treating hospital, which included their name, and their study data. In particular, those responsible for the analysis of the samples for mercury did not know whether a child had been given succimer or placebo.

We measured whole blood total mercury in all tested samples. Total mercury consists of inorganic mercury and organic mercury. In the US, 80%–95% of mercury in blood is methyl mercury.[29] Since the lab's experience was that inorganic mercury was not detected in samples with less than 1 µg/L total mercury, we measured inorganic mercury in samples in which the total mercury concentration was ≥ 1 µg/L. In addition, if the baseline samples were measured for inorganic mercury, the post-treatment samples were also measured for it regardless of the total mercury concentration. Specimens were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) for total mercury and automated cold vapor atomic absorption spectrophotometry (CVAAS) for inorganic mercury.[30] The limit of detection (LOD) was 0.33 µg/L for total mercury and 0.35 µg/L for inorganic mercury. We calculated organic mercury as total mercury minus inorganic mercury.

For both total mercury and inorganic mercury measures, National Institute of Standards and Technology Standard Reference Material 966 was used as a bench quality control material as well as 3 levels of in-house blood pools traceable to the reference material for daily quality control. One of 2 different levels of a blind quality-control material was inserted in every analytical group of samples for an additional quality control check. All results of mercury given in µg/L can be converted to nmol/L by multiplying by 4.99.

Statistical Analysis

We used an intention-to-treat analysis in General Linear Models (GLMs). Values $< \text{LOD}$ were replaced with $1/2 \text{ LOD}$ in the models. The distributions of total mercury and organic mercury concentrations were positively skewed; therefore, we did a logarithmic transformation of these variables so that the data were approximately homoscedastic (i.e., had equal variances) among test groups and were also approximately normally distributed. Comparisons of mercury concentrations between treatment and placebo groups at baseline, one week after treatment initiation, and 5 months after treatment initiation, when all three courses of treatment were completed, were made in the GLM with adjustment for exact age at mercury measurement, sex, race, and clinical center. The adjusted geometric mean (GM) of blood mercury concentrations and 95% confidence intervals (CIs) at each time point for each group were also estimated by GLM. We investigated the trend over time in the mean mercury concentrations in the succimer group relative to those of the placebo group. We tested the hypothesis that there was a monotonic trend in the difference between the adjusted mean mercury concentrations in the succimer group and the placebo group. We performed this test using a bootstrap-based isotonic regression method as described in the Appendix. This method accounts for dependence within-subject, and adjusts for age, sex, center, race

and the mercury concentrations at the previous time point.[31, 32] We used SAS 9.13 (SAS Institute, Inc., Cary, NC) for GLM analyses.

RESULTS

A total of 780 children were randomized, with 396 children allocated to active drug and 384 allocated to placebo. Of children receiving succimer, 83% required retreatment after the first course, and 83% of those receiving a second course of treatment required a third.[27] The trial participant flow and the number of children completing the treatment at each course are shown in Figure 1, which is a standard flow chart for the whole trial with the numbers of children having mercury analyses added.

The recruitment period spanned 3 years, from 1994 to 1997. By parents' reports, over 90% of the assigned doses of study drug were given, and by pill count, about 76% of the capsules were gone from the bottle. Forty percent of the families of children given succimer and 26% of the families of children given placebo reported difficulty administering the drug ($p<0.01$). Interruptions in the administration of the drug occurred at similar rates in the two groups (30% with succimer vs. 27% with placebo, $p=0.4$).[27]

Blood samples were collected for all 780 children at baseline and 13 of them were excluded from the present analysis because of a problem with the stored samples. Total mercury was measured in these 767 samples (393 given succimer and 374 placebo) and detected and quantified in 657 (86%) samples (338 succimer and 319 placebo). Inorganic mercury was analyzed in 143 baseline samples (76 succimer and 67 placebo) and 42 (29%) had detectable amounts (19 succimer and 23 placebo).

At one week after initiation of treatment, blood samples were collected for 778 children. Total mercury was measured in 768 samples (389 succimer and 379 placebo) and detected and quantified in 623 (81%) samples (313 succimer and 310 placebo). Inorganic mercury was analyzed in 143 samples (72 succimer and 71 placebo) and 57 (40%) had a detectable concentration (30 succimer and 27 placebo).

Of 338 children completing 3 courses of succimer, we took a 20% random sample for mercury analysis. Of these 70 children (35 given succimer and 35 placebo), 3 were excluded because of sample problems. Total mercury was detected and quantified in 61 of 67 (91%) samples (30 succimer and 31 placebo). Inorganic mercury was analyzed in 18 samples and 5 (28%) had detectable concentration (2 succimer and 3 placebo).

Because inorganic mercury was found in fewer than 8% of total samples, we used it only to provide a more precise estimate of organic mercury by subtracting it from total mercury, and we do not address findings related to inorganic mercury further. Although we report here the results for organic mercury, the results for total mercury are very similar (data not shown).

At baseline, the mean organic mercury concentrations of the succimer group were about 99% of the concentrations of the control group (Table 2). At one week after treatment was begun, organic mercury concentration stayed the same in the placebo group but decreased 8% in the succimer group. The difference between the placebo group and the succimer group was statistically significant ($p=0.04$). After 3 courses of treatment, which took about 5 months, the mean organic mercury concentrations in the succimer group were about 80% of the levels of the control group. The difference did not appear to arise from a reduction in the succimer group, but rather from prevention of the increase over time in the placebo group (Figure 2). The isotonic regression trend analysis showed that the difference between the succimer and placebo group increased over time, with a trend p -value of 0.048. We repeated this analysis using only the 67 children from the 20% random sample who completed all 3

courses. At baseline, the mercury concentration in the succimer group was 79% of that in the placebo group (0.45 vs 0.57 µg/L); at one week, it was 66% (0.45 vs 0.68 µg/L); after 3 courses, it was 87% (0.54 vs 0.62 µg/L). The trend test for an increasing difference is statistically significant ($p < 0.05$), but the concentration in the succimer group still increases.

DISCUSSION

In a randomized trial in children aged 12 to 33 months, we found that succimer treatment produced a modest reduction in organic mercury concentration at one week, and slowed or prevented but did not reverse accumulation of organic mercury after multiple courses over 5 months. This is the largest study of succimer (or any chelating agent) and mercury in children, and the only one to include randomized controls. In the parent study of succimer for lead poisoning, succimer produced a much larger (42%) difference (placebo 24 µg/dL vs. succimer 14 µg/dL) in blood lead concentration after one week of therapy.[27] Although some of this is because the children were selected for high blood lead concentrations, it appears that succimer is a less effective chelator for organic mercury than for lead. This may be because the succimer-lead complexes are more stable than succimer-mercury complexes. [33]

Although mercury concentrations in this study are low, they are 70% higher than in the NHANES children (0.56 µg/L vs. 0.33–0.34 µg/L). According to Schober's and the CDC's reports, blood mercury concentration was significantly higher in African-American NHANES participants (>0.50 µg/L vs 0.27–0.45 µg/L in whites).[34, 35] Most children in the TLC study are non-Hispanic black (77%) and it may partially explain the higher mercury concentration in the TLC children than in the NHANES children, who were 22% black.

The dose of succimer used in TLC was based on body surface area, which yielded higher doses for these 25 month old children than would have been used if dose were calculated by weight. In addition, TLC used a higher loading dose for the first week of its 26 day courses of therapy, and, based on pill count and decreasing blood lead concentration, demonstrated adherence as good as has been seen in shorter, less intense trials in children. [36, 37] Succimer is a difficult drug to administer to young children. It has an unpleasant "rotten egg" odor, the capsules must be opened and the contents sprinkled onto apple sauce, pudding or other palatable vehicle, and it must be given 3 times per day. Thus, because of the high adherence to succimer in the TLC study, and relatively higher doses used based on the body surface area algorithm used to calculate dosage, it seems unlikely that the small effect that we found in mercury reduction from succimer administration could be improved upon by larger doses or extended courses.

The study limitations include the small number (8%) of children with detectable inorganic mercury, so we can draw no conclusions about inorganic mercury. We did not investigate the sources of the mercury exposure of the TLC children, although previous studies show that methyl mercury in children comes from food, especially fish.[38] We do not know whether succimer treatment changed fish consumption in the study subjects, but that change is unlikely to underestimate the efficacy of succimer at these mercury levels. In the TLC study, children were followed until age 7, and we have already reported that succimer does not improve IQ or behavioral test scores in these children.[28] Thus, even if succimer does prevent accumulation of organic mercury, it does not prevent any effects of organic mercury on the tests we did. Finally, although of no direct relevance to specific neurobehavioral conditions, we have analyzed the TLC data for intelligence and behavior incorporating these mercury data, and found no deleterious effect of baseline total or organic mercury.[39]

CONCLUSION

Succimer may lower organic mercury blood concentrations modestly from original levels of about 0.5 µg/L, and with months of treatment will slow or prevent accumulation. These small changes seem unlikely to produce any clinical benefit.

Acknowledgments

This study was supported by the NIEHS Intramural Research Program, the National Center for Minority Health and Health Disparities at NIH, and the Center for Environmental Health at CDC. Chemet® and placebo were gifts from McNeil Labs, Fort Washington PA, USA

Appendix: Trend test for the difference in the mean organic mercury levels between treatment and control groups

Suppose the mean mercury levels (log-scale) at the t^{th} time ($t = 1$ for baseline, $t = 2$ for 1 week and $t = 3$ for 5 months) for the control and treatment groups are denoted as $\mu_t^{control}$ and $\mu_t^{treatment}$, respectively. Let $\Delta_t = \mu_t^{treatment} - \mu_t^{control}$ denote the difference in the mean organic mercury levels between the two groups at time t . Then we want to test:

$$H_0: \Delta_1 = \Delta_2 = \Delta_3 \text{ Versus}$$

$$H_a: \Delta_1 \geq \Delta_2 \geq \Delta_3, \text{ with at least one strict inequality among the parameters.}$$

Note that at baseline we expect no difference between the two groups, that is $\Delta_1 = 0$, but over time we expect Δ_t to become more negative.

Since the mercury levels depend on some covariates such as the current age of the child, sex, race, center, and the mercury concentration at the previous time point, we estimated each Δ_t using least squares means derived from linear regression model, by adjusting for these covariates. Statistical procedure PROC GLM in SAS (version 9.13) was used for this purpose. Denote these estimated values by $\hat{\Delta}_t$. Using these estimates, we then constructed the isotonic regression estimators for Δ_t and accordingly constructed a likelihood ratio type statistic.[31] To derive the p-value, we employed bootstrap methodology which is a nonparametric procedure.[32] Since the mean change in mercury levels depends upon various covariates, we bootstrapped the residuals within each group at each time point. Secondly, since we have a longitudinal data, we resampled the subjects. That way we retain the underlying correlation structure in the data. The null mean for the bootstrap was taken to be the average of $\hat{\Delta}_t$ across the time points. The bootstrap distribution under the null hypothesis was derived using 10,000 bootstrap samples.

Abbreviations

CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
DMPS	Dimercaptopropane sulfonic acid
DMSA	Dimercaptosuccinic acid
GLM	General Linear Model
LOD	limit of detection
TLC	Treatment of Lead-exposed Children

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Figure 1.
Flow of patients through the TLC clinical trial.

Figure 2.

Mean blood concentration of organic mercury in children given succimer or placebo. Points are at about one week prior to treatment, after one week of treatment, and after ~20 weeks, when 3 courses of treatment were complete. Numbers indicate the number of children at each point. Whiskers are 95% confidence intervals; only one side is shown for clarity, but they are symmetric.

Table 1

Comparison of baseline demographic characteristics and blood lead concentrations of children assigned to receive succimer or placebo from 1994 through 1997. Values are numbers (percentage) or meansstandard deviation (sample size) unless stated otherwise.*

Characteristic	Placebo	Succimer	p value
Ethnic group or race			0.44
White	42(11)	46(12)	
African American	292(76)	310(78)	
Other	50(13)	40(10)	
Female	166(43)	179(45)	0.58
English-speaking	364(95)	377(95)	0.79
Parent's education			0.86
<12 yrs	155(40)	161(41)	
=12 yrs	165(43)	164(41)	
>12 yrs	64(17)	71(18)	
Neither parent working	220(57)	234(59)	0.55
Living with single parent	279(73)	282(72)	0.73
Annual family income			0.63
<\$10,000	137(36)	152(38)	
≥\$10,000	102(27)	107(27)	
Unknown	145(38)	137(35)	
Age (months)	25±6 (384)	24±6 (396)	0.79
Height (cm)	86±6(384)	86±6(396)	0.61
Weight (kg)	12±2(384)	12±2(396)	0.96
Body surface area (m ²)	0.5±0.1(384)	0.5±0.1(396)	0.82
Baseline blood lead (µg/dL)	26±5(384)	27±5(396)	0.14

* Rates were compared by Pearson chi-square test and means were compared by t test with equal variances.

Table 2

Comparison of baseline, 1-week and 5-month organic mercury concentrations between the placebo group and the succimer group.

Time	Placebo *	Succimer *	P value **	Ratio estimate ^{†,‡} (Succimer relative to Placebo)
Baseline blood mercury (µg/L)	0.53, n=374 (0.49, 0.57)	0.52, n=393 (0.49, 0.56)	0.72	0.99 (0.90, 1.08)
1-week blood mercury (µg/L)	0.52, n=379 (0.49, 0.55)	0.48, n=389 (0.47, 0.51)	0.04	0.93 (0.87, 1.00)
5-month blood mercury (µg/L)	0.67, n=33 (0.55, 0.82)	0.55, n=34 (0.43, 0.70)	0.19	0.82 (0.61, 1.11)

* Geometric mean and 95% CI, adjusted for concurrent age, sex, race, center and the organic mercury concentration at the previous time point.

** P values for the difference between the means (in log-scale) of organic mercury of the Placebo and the Succimer groups at each individual time point, after adjusting for concurrent age, sex, race, center and the organic mercury concentration at the previous time point.

[†] Ratio of mean organic mercury of the Succimer group to that of the Placebo group, after adjusting for concurrent age, sex, race, center and the organic mercury concentration at the previous time point.

[‡] The trend in the ratio of mean organic mercury of Succimer group to that of the Placebo group, after adjusting for concurrent age, sex, race, center and the organic mercury concentration at the previous time point, is significant (P = 0.048). Thus, there is a significantly increasing trend of reduction in mean mercury levels.