Efficacy of Succimer Chelation of Mercury at Background Exposures in Toddlers: A Randomized Trial

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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 levels in pre-treatment samples from 767 children. We also measured mercury levels 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 with time in the difference between the adjusted mean mercury concentrations in the succimer group and that in 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 3 courses of treatment (*P* for trend = .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. *(J Pediatr 2011;158:480-5)*.

Children can be exposed to metallic mercury and its vapor from a variety of sources. Methyl mercury is a common food contaminant.[1] High exposures to elemental or inorganic mercury produce acrodynia.[2] High prenatal exposure to methyl mercury produces a cerebral palsy-like illness in children.[3-5] 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] Two large prospective studies of children with relatively high prenatal exposures have been in progress, one in the Seychelle Islands[8] and the other in the Faroe Islands.[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 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 that for influenza beginning in 1999. Although there is scientific consensus rejecting a causal relationship between thimerosal-containing vaccines and autism,[13] this has not translated into public opinion.

Although chelating drugs can remove mercury from the body and prevent further deterioration in acute situations,[14] they have not been shown to reverse damage to the central nervous system or improve neuropsychological functions.[15-17] In experimental studies, succimer (meso-2,3-dimercapto-succinic acid or DMSA)[18] did not remove methyl mercury from the brain of poisoned rodents,[19] nor did it increase the urinary excretion of methyl mercury in rodents[20] or in persons who consume presumably contaminated sport fish.[21] 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 body mercury burden in the hope of reducing some autism symptoms. Anecdot-

GLM	General linear model
LOD	Limit of detection
NHANES	National Health and Nutrition Examination Survey
TLC	Treatment of Lead-exposed Children

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ally, succimer is used for this, although this practice is based on only one small uncontrolled study[22] that has not been accepted as evidence of safety or efficacy.[23] 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 because of safety concerns and lack of evidence for direct benefit to participants.[24]

We have completed a randomized clinical trial of succimer for lead poisoning in 780 children aged 12 to 33 months, called the Treatment of Lead-exposed Children (TLC) trial.[25-27] The samples remaining from this study have allowed us to study the effect of succimer in reducing blood mercury concentrations in toddlers and thus to fill a gap in the scientific literature that is unlikely to be addressed any other way.

Methods

The blood samples and data come from the TLC study, a 4-site, placebo-controlled randomized trial, 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 μg/dL. Children who had confirmed venous blood lead concentrations between 20 and 44 μg/dL and lived in cleanable housing (by vacuuming, damp mopping, or wiping to minimize lead exposure) had a second screening visit approximately 1 week later.[25] When the blood lead concentration at the second visit was also between 20 and 44 μg/dL, the children entered the randomization phase, and their houses were 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 this report, we constructed a data set that included demographics, treatment information, and mercury levels, but not personal identifiers. We applied for and received a human subjects research exemption for this analysis from the Office of Protection from Research Risks at the National Institutes of Health.

Treatment assignments were randomized within the strata of the 4 clinical centers, 6 categories of body surface area, two strata of blood lead concentrations (≤25 μg/dL or >25 μg/dL) and languages (English or Spanish).[25] Children could receive as many as 3 courses of succimer or placebo. McNeil Consumer Products (Fort Washington, Pennsylvania) provided unmarked succimer (Chemet, 100 mg) and placebo capsules of identical appearance. The dose was calculated on a body surface area basis.[25] 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 ≥15 μg/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 re-treatment to match the frequency of re-treatment of children given succimer within the blocks used in the initial randomization.[25]

Measurement of Mercury

For this 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 the Centers for Disease Control analyzed all blood samples drawn approximately 1 week before randomization (baseline) and 1 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 3 courses. The identification numbers of samples were re-coded by the data-coordinating center to de-link the association to the child's clinical record at the treating hospital. In particular, researchers 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 concentration (inorganic mercury and organic mercury) in all tested samples. In the United States, 80% to 95% of mercury in blood is methyl mercury.[28] Because the laboratory's experience was that inorganic mercury was not detected in samples with <1 μg/L total mercury, we measured inorganic mercury in samples in which the total mercury concentration was ≥1 μg/L. In addition, when 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 by using inductively coupled plasma mass spectrometry for total mercury concentration and automated cold vapor atomic absorption spectrophotometry for inorganic mercury concentration.[29] The limit of detection (LOD) was 0.33 μg/L for total mercury concentration and 0.35 μg/L for inorganic mercury concentration. 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 concentrations 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 less than the LOD were replaced with half the 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 (ie, had equal variances) in test groups and were also approximately normally distributed. Comparisons of mercury concentrations between treatment and placebo groups at baseline, 1 week after treatment initiation and 5 months after treatment initiation, when all 3 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 of blood mercury concentrations and 95% CIs at each point for each group were also estimated with GLM. We investigated the trend with 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 that in the placebo group. We performed this test with a bootstrap-based isotonic regression method, as described in the **Appendix** (available at www.jpeds.com). This method accounts for dependence within-subject and adjusts for age, sex, center, race, and the mercury concentrations at the earlier point.[30,31] We used SAS software version 9.13 (SAS Institute, Cary, North Carolina) for GLM analysis.

Results

A total of 780 children were randomized, with 396 children allocated to active drug and 384 allocated to placebo. Of the children receiving succimer, 83% required re-treatment after the first course, and 83% of those receiving a second course of treatment required a third.[26] 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. According to parents' reports, >90% of the assigned doses of study drug were given, and by using pill count, approximately 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 ($P < .01$). Interruptions in the administration of the drug occurred at similar rates in the two groups (30% with succimer versus 27% with placebo, $P = .4$).[26]

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

One week after initiation of treatment, blood samples were collected for 778 children. Total mercury concentration was measured in 768 samples (389 succimer and 379 placebo) and detected and quantified in 623 samples (81%; 313 succimer and 310 placebo). Inorganic mercury was analyzed in 143 samples (72 succimer and 71 placebo), and 57 samples (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 succimer group and 35 placebo group), 3 were excluded because of sample problems. Total mercury was detected and quantified in 61 of 67 samples (30 succimer and 31 placebo). Inorganic mercury was analyzed in 18 samples, and 5 samples (28%) had detectable concentration (2 succimer and 3 placebo).

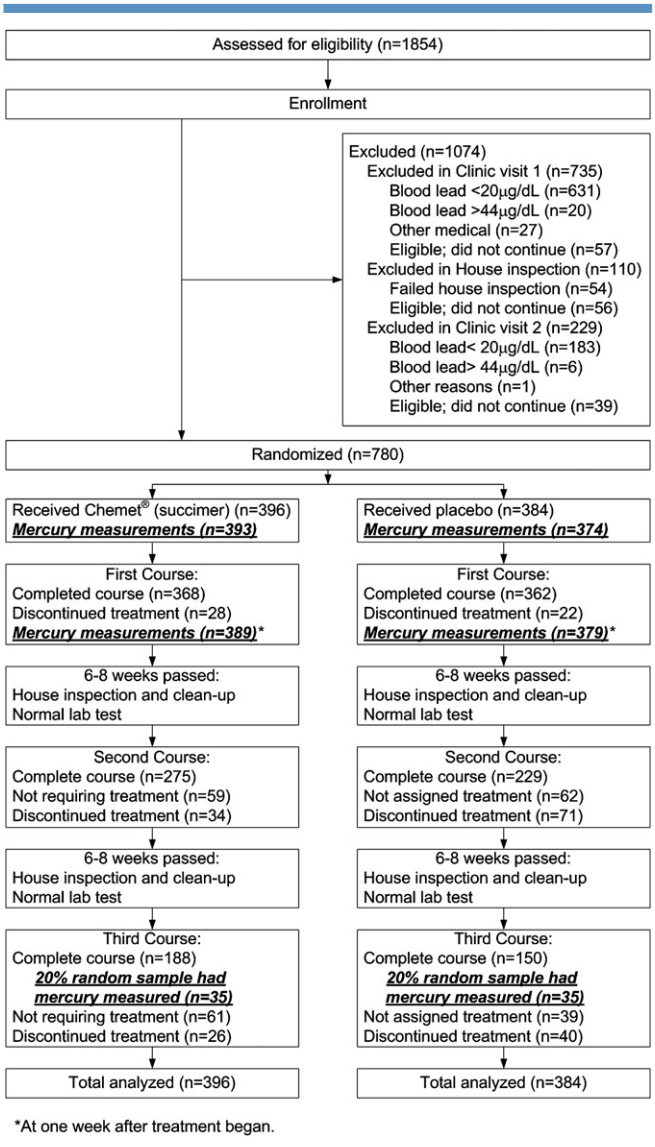

Figure 1. Flow of patients through the TLC clinical trial.

Because inorganic mercury was found in <8% of the 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).

The baseline characteristics were balanced in two groups (**Table I**). At baseline, the mean organic mercury concentrations of the succimer group were approximately 99% of the concentrations of the control group (**Table II**). One week after treatment began, organic mercury

Table I. Comparison of baseline demographic characteristics and blood lead concentrations of children assigned to receive succimer or placebo from 1994 through 1997

Characteristic	Placebo	Succimer	*P* value
Ethnic group or race			.44
Caucasian	42 (11)	46 (12)	
African-American	292 (76)	310 (78)	
Other	50 (13)	40 (10)	
Female	166 (43)	179 (45)	.58
English-speaking	364 (95)	377 (95)	.79
Parent's education			.86
<12 years	155 (40)	161 (41)	
12 years	165 (43)	164 (41)	
>12 years	64 (17)	71 (18)	
Neither parent working	220 (57)	234 (59)	.55
Living with single parent	279 (73)	282 (72)	.73
Annual family income			.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)	.79
Height (cm)	86 ± 6 (384)	86 ± 6 (396)	.61
Weight (kg)	12 ± 2 (384)	12 ± 2 (396)	.96
Body surface area (m^2)	0.5 ± 0.1 (384)	0.5 ± 0.1 (396)	.82
Baseline blood lead (μg/dL)	26 ± 5 (384)	27 ± 5 (396)	.14

Values are numbers (percentage) or means ± SD (sample size), unless stated otherwise. Rates were compared with Pearson χ^2 test and means were compared with *t* test with equal variances.

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 ($P = .04$). After 3 courses of treatment, which took approximately 5 months, the mean organic mercury concentrations in the succimer group were approximately 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 in time in the placebo group (Figure 2). With the isotonic regression trend analysis, the difference between the succimer and placebo group was shown to increase in time, with a trend *P* value of .048. We repeated this analysis with 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 versus 0.57μg/L); at 1 week, it was 66% (0.45 versus 0.68 μg/L); after 3 courses, it was 87% (0.54 versus 0.62 μg/L). The trend test for an increasing difference is statistically significant ($P < .05$), but the concentration in the succimer group still increases. The statistical significance of the trend is likely due to the large difference at week 1.

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 1 week and slowed or prevented, but did not reverse, accumulation of organic mercury after multiple courses in 5 months. This is the largest study of succimer (or any chelating agent) and mercury in children, and the only one to include randomized control subjects. In the parent study of succimer for lead poisoning, succimer produced a much larger (42%) in blood lead concentration difference (placebo 24 μg/dL versus succimer 14 μg/dL) after 1 week of therapy.[26] 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.[32]

Although mercury concentrations in this study are low, they are 70% higher than in the children in National Health and Nutrition Examination Survey (NHANES; 0.56 μg/L versus 0.33-0.34 μg/L). According to Schober's and the Centers for Disease Control's reports, blood mercury concentration was significantly higher in African-American NHANES participants (>0.50 μg/L versus 0.27-0.45μg/L in Caucasian participants).[33,34] Most children in the TLC study were non-Hispanic African-American (77%), and this may partially explain the higher mercury concentration in the children in the TLC study compared with that in the children in NHANES, who were 22% African-American.

The dose of succimer used in the TLC study 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, the TLC study used a higher loading dose for the first week of its 26-day courses of therapy and, on the basis of pill count and decreasing blood lead concentration, demonstrated adherence as good as has been seen in shorter, less intense trials in children.[35,36]

Table II. 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)	.72	0.99 (0.90-1.08)
1-week blood mercury (μg/L)	0.52, n = 325 (0.49-0.55)	0.48, n = 336 (0.47-0.51)	.04	0.93 (0.87-1.00)
5-month blood mercury (μg/L)	0.67, n = 33 (0.55-0.82)	0.55, n = 31 (0.43-0.70)	.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 earlier point.
†*P* values for the difference in the means (in log-scale) of organic mercury of the placebo and the succimer groups at each individual point, after adjusting for concurrent age, sex, race, center, and the organic mercury concentration at the earlier 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 earlier 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 earlier point, is significant ($P = .048$). Thus, there is a significantly increasing trend of reduction in mean mercury levels.
¶n: number of complete observations without missing value in covariates.

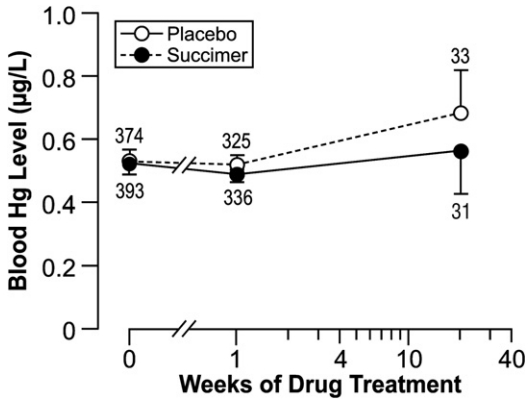

Figure 2. Mean blood concentration of organic mercury in children given succimer or placebo. Points are at approximately 1 week before treatment, after 1 week of treatment, and after approximately 20 weeks, when 3 courses of treatment were complete. Numbers indicate the number of complete observations without missing values for covariates. Whiskers are 95% CIs; only one side is shown for clarity, but they are symmetric.

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 applesauce, 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 on the basis of 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 with larger doses or extended courses.

The study limitations include the small number of children with detectable inorganic mercury (8%), so we can draw no conclusions about inorganic mercury. We did not investigate the sources of the mercury exposure of the children in the TLC study, although earlier studies show that methyl mercury in children comes from food, especially fish.[37] 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 years, and we have already reported that succimer does not improve IQ or behavioral test scores in these children.[27] Thus, even if succimer does prevent accumulation of organic mercury, it does not prevent any effects of organic mercury on the tests we conducted. Finally, although it is 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.[38]

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

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Appendix

Trend Test for the Difference in the Mean Organic Mercury Levels between Succimer Group and Placebo Group

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 placebo and succimer groups are denoted as $\mu_t^{placebo}$ and $\mu_t^{succimer}$, respectively. Let $\Delta_t = \mu_t^{succimer} - \mu_t^{placebo}$ denote the difference in the mean organic mercury levels between the two groups at time t. Then we want to test:

$H_0 : \Delta_1 = \Delta_2 = \Delta_3$ Versus

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

At baseline, we expect no difference in the two groups, that is $\Delta_1 = 0$, but with time, we expect Δ_t to become more negative.

Because the mercury levels depend on some covariates such as the current age of the child, sex, race, center, and the mercury concentration at the earlier time point, we estimated each Δ_t by using least squares means derived from linear regression model, adjusting for these covariates. Statistical procedure PROC GLM in SAS software (version 9.13) was used for this purpose. We denote these estimated values with $\widehat{\Delta}_t$. By 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 used bootstrap methodology, which is a nonparametric procedure.[32] Because the mean change in mercury levels depends on various covariates, we bootstrapped the residuals within each group at each time point. Second, because we had a longitudinal data, we re-sampled the subjects. That way, we retained the underlying correlation structure in the data. The null mean for the bootstrap was taken to be the average of $\widehat{\Delta}_t$ across the time points. The bootstrap distribution with the null hypothesis was derived by using 10 000 bootstrap samples.