Oral Succimer Decreases the Gastrointestinal Absorption of Lead in Juvenile Monkeys

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Although succimer (Chemet, *meso*-2,3-dimercaptosuccinic acid, DMSA) is considered to be a safe and effective chelating agent for the treatment of lead poisoning in humans, there is concern that it may increase the gastrointestinal (GI) absorption and retention of Pb from exposures suffered concurrent with treatment. This concern is justified because the availability of Pb-safe housing during outpatient treatment with oral succimer is limited. We used a juvenile nonhuman primate model of moderate childhood Pb intoxication and a sensitive double stable Pb isotope tracer methodology to determine whether oral succimer chelation affects the GI absorption and whole-body retention of Pb. Infant rhesus monkeys (n = 17) were exposed to Pb daily for 1 year postpartum to reach and maintain a target blood lead (BPb) level of 35–40 µg/dL. Animals were administered succimer (n = 9) or vehicle (n = 8) over two successive 19 day succimer treatment regimens beginning at 53 and 65 weeks of age. The present study was conducted over the second chelation regimen only. Animals received a single intravenous (iv) dose of stable ^{204}Pb tracer (5 µg, 24.5 nmol) followed by a single oral dose of stable ^{206}Pb tracer (72.6 µg, 352 nmol) immediately before chelation, in order to specifically evaluate GI Pb absorption and whole-body Pb retention with treatment. We collected complete urine and fecal samples over the first 5 days and whole blood over the first 8 days of treatment for analyses of stable Pb isotopes using magnetic sector inductively-coupled plasma mass spectrometry. Results indicate that succimer significantly reduced the GI absorption of Pb (vehicle, 64.9% ± 5.5; succimer, 37.0% ± 5.8; mean ± SEM). Succimer also significantly increased the urinary excretion of endogenous Pb by approximately 4-fold over the vehicle treatment, while endogenous fecal Pb excretion was decreased by approximately 33%. Finally, although succimer reduced the whole-body retention of endogenous Pb by approximately 10% compared to vehicle, the majority (77%) of the administered internal dose of Pb tracer was retained in the body when assessed after 5 days of treatment. These data do not support the concern that succimer treatment increases GI Pb absorption. *Key words*: 2,3-dimercaptosuccinic acid, absorption, chelation, Chemet, lead, lead exposure, nonhuman primate, succimer. *Environ Health Perspect* 109:613–619 (2001). [Online _____]
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Research continues to identify adverse effects of lead at progressively lower levels of exposure, thereby increasing the number of children estimated to be at risk for cognitive impairment (*1–4*). Further, public health programs have achieved limited success in reducing Pb exposures through primary prevention, despite the recognized importance of this goal (*5*). This underscores the need to develop improved intervention strategies, particularly for moderately Pb-poisoned children who have blood Pb (BPb) levels between 20 and 45 µg/dL.

Succimer [Chemet, *meso*-2,3-dimercaptosuccinic acid, DMSA] is considered to be a safe and effective chelating agent for the treatment of Pb poisoning in humans (*6–9*). However, there remain a number of outstanding questions that should be addressed to better evaluate the overall utility of succimer treatment for moderately Pb-poisoned children. For example, the effects of succimer on the gastrointestinal (GI) absorption and retention of Pb from exposures suffered concurrently with treatment are not well known. Effects of oral succimer on GI Pb absorption have ranged from no detectable effect in humans (*8*) to a decrease in rats (*10*). Other Pb-chelating agents [$CaNa_2EDTA$, citrate, penicillamine, and 2,3-dimercapto-1-propanol, also known as British Anti-Lewisite (BAL)] have been shown to increase GI Pb absorption, and in some cases (citrate, penicillamine, and BAL) this has resulted in an increase in body Pb burden (*11*).

Concern over the effects of oral succimer on GI lead absorption is justified because succimer exhibits high Pb-binding affinity [Pb-DMSA chelate formation constant = 2.9 × 10^{17} (*12*)]. Also, the potential exists for continued elevated, albeit lower, Pb exposures during outpatient treatment with oral succimer. This is because environmental Pb exposures are often difficult to identify and effectively eliminate (*13,14*) and because the availability of Pb-safe housing during outpatient chelation treatment is often limited.

Here we used a juvenile nonhuman primate model of moderate childhood Pb intoxication and a sensitive double stable Pb isotope tracer methodology to determine whether oral succimer chelation affects the GI absorption and whole-body retention of Pb. This investigation was a substudy of a larger study that investigated the efficacy of succimer chelation therapy for reducing tissue Pb concentrations and alleviating cognitive impairment. Results from that larger study have been reported elsewhere (*15–17*) or are forthcoming.

Materials and Methods

Study design/treatments. The present study was composed of a 1 × 2 factorial design (Pb exposed × vehicle or succimer; n = 8–9/group). Animals were exposed to Pb daily for 1 year to achieve and maintain target BPb levels of 35–40 µg/dL; animals were then treated with two successive 19 day regimens of succimer or vehicle starting at 53 and 65 weeks of age. The present study was conducted during the second chelation at 65 weeks of age. Thus, daily Pb exposure in these animals ceased 12 weeks before this study was conducted.

Animals. Seventeen female infant rhesus monkeys (*Macaca mulatta*) born at the Harlow Center for Biological Psychology breeding colony were used in this study. We assigned animals to vehicle (n = 8) and succimer (n = 9) treatment groups at birth. Complete details of the animal recruitment and care procedures are reported elsewhere (*15,17*). Briefly, the infants were housed in a single cage with their mothers until weaning at age 26 weeks; they were subsequently housed in groups of five females and one nonstudy male in group cages. After weaning, the monkeys were maintained on Purina Monkey Chow (#5037; PMI Feeds, Inc., St. Louis, MO) *ad libitum*. All procedures related to animal care conformed to the

guidelines set forth in the *Guide for the Care and Use of Laboratory Animals* (*18*).

Lead exposure. We orally administered lead acetate (Fisher Chemical, Fairlawn, NJ) to the infant monkeys in a solution of Similac with iron formula (Abbott Laboratories, Columbus, OH) daily between 1430 and 1630 hr beginning on day 8 postpartum through age 26 weeks. After weaning the monkeys received their oral Pb dose dissolved in apple juice. The required dose of Pb acetate was dissolved in 50% glucose before being added to the Similac or apple juice. Lead was administered daily in amounts targeted to produce BPb levels of approximately 35–40 µg/dL. The Pb dose was adjusted biweekly as needed to maintain the target BPb level. Oral Pb exposure was discontinued immediately before the first succimer chelation period (i.e., at the end of 52 weeks of age). Thus, daily Pb exposure to the animals ceased 12 weeks before this study was conducted. The target BPb level of 35–40 µg/dL was chosen because it is within the range of BPb levels at which succimer is being used in children, and it is also within the range of the BPb levels in children (20–45 µg/dL) participating in the succimer clinical trials (*19*). In previous studies, we observed no overt signs of Pb toxicity (e.g., decreased weight gain, reduced appetite, lethargy) in infant rhesus monkeys at chronic BPb levels of 30–80 µg/dL (*20,21*).

Administration of intravenous ^{204}Pb and oral ^{206}Pb stable isotope tracers. All animals received 5 µg (24.5 nmol) of ^{204}Pb stable isotope tracer via a single intravenous (iv) injection within approximately 30 min before beginning chelation treatment. The stable ^{204}Pb tracer (^{204}PbCO$_3$, 99.71% ^{204}Pb enriched; National Institute of Standards and Technology, Gaithersburg, MD) was prepared by dissolving ^{204}PbCO$_3$ to a concentration of 1,000 µg ^{204}Pb/mL in ultrapure 1N HNO$_3$. An aliquot of this solution was evaporated to dryness and redissolved in acidified (pH 2) sterile ultra-pure water (> 18 Mohm-cm^2) to a concentration of 100 µg ^{204}Pb/mL. For administration to the animals, we diluted this solution into 1 mL sterile saline (final pH 7.0) immediately before dosing. The Pb tracer vial was rinsed twice with 1 mL aliquots of sterile saline and the entire contents (3 mL) administered to the animal.

A single oral dose of 72.6 µg (352 nmol) ^{206}Pb tracer was administered orally by syringe within approximately 15 min after the iv ^{204}tracer dose. The stable ^{206}Pb tracer (^{206}PbCO$_3$, 99.66% ^{206}Pb enriched, National Institute of Standards and Technology, Gaithersburg, MD) was prepared as described above, except that apple juice was used in place of sterile saline as the carrier/diluent. Following delivery of the 3 mL dose of tracer, the syringe was washed three times with apple juice and the rinses given orally to ensure that all the tracer was administered, resulting in a total delivery of 12 mL apple juice. Food was withheld for 20 hr before and 1.5 hr after administration of the oral ^{206}Pb dose to minimize any confounding effects of the fed state on GI Pb absorption.

This double stable Pb isotope tracer method provided a means to accurately determine both the GI absorption and the whole-body retention of Pb. The administered ^{206}Pb tracer was used to determine the GI absorption of Pb. We used the ^{204}Pb tracer to correct for the excretion of endogenous Pb into the feces (e.g., biliary) that would otherwise confound the measurement of GI Pb absorption based on the collection of oral ^{206}Pb tracer eliminated in the feces. We also used the administered ^{204}Pb tracer and the absorbed oral ^{206}Pb tracer to evaluate the whole-body retention of Pb. Notably, data on the use of this tracer method in these animals has demonstrated no measurable difference in the chelation of the ^{204}Pb tracer versus Pb absorbed from chronic oral exposures (*17*). This suggests that iv-administered stable Pb isotope accurately reflects the fate of chelatable "total" Pb in the body. For clarity in this paper, we refer to body Pb accumulated from the chronic oral exposures administered over the first year of life as "inherent" body Pb.

Succimer treatment. Succimer (as Chemet) was administered following manufacturers instructions and standard clinical procedures (*7*). Treatment with oral succimer or vehicle began at a mean age of 53 weeks (first chelation) and again at 65 weeks (second chelation). The present study was conducted over the second chelation regimen. Succimer was administered at a dose of 30 mg/kg/day divided into three doses per day for 5 days (administered at 0900, 1600, and 2300 hr); treatment continued for an additional 14 days at 20 mg/kg/day divided into two doses per day (administered at 0900 and 2300 hr), for a total 19 day treatment regimen. Succimer dosing commenced within approximately 15 min after administration of the oral ^{206}Pb tracer dose. Succimer was orally administered in apple juice vehicle via syringe within 15 min of dissolving the succimer to ensure maximum activity. The vehicle group received succimer placebo that was obtained from the pediatric clinical trials (*19*) and administered as described above for succimer.

Sample collection. All sample collection, storage, and laboratory ware (i.e., Teflon, polyethylene, polypropylene, and stainless steel) were acid-cleaned using established procedures (*22*). Sample processing and analyses were performed using reagents that were double sub-boiling quartz-distilled, high-purity grade, and ultrapure water (> 18 Mohm-cm^2).

Blood. Whole blood samples (0.5–1 mL) were collected between 0900 and 1100 hr by femoral venipuncture into heparinized Vacutainer tubes (#367734; Becton-Dickinson, Franklin Lakes, NJ) and stored frozen until analyzed. Samples were collected from all animals on days 0, 1, 3, 5, and 8 relative to the start of chelation on day 0.

Urine and feces. One day before the start of chelation, individual animals were placed in stainless steel metabolic cages, where they remained throughout the first 5 days of treatment (for a total of 6 consecutive days) in order to facilitate complete urine and fecal collections. Except for the overnight food fast before receiving tracer, animals were given free access to food and water while in the metabolic cages. Urine collection bottles were changed and feces collected at 24-hr intervals.

The metabolic cages were modified for this study to minimize the transfer of food and fecal material into the collected urine sample (*16*). To minimize sample contamination with Pb, cages were cleaned daily by rinsing with ultrapure water and weekly (i.e., between placing new animals in the cage) using soap and water, acid-washing with dilute HNO$_3$, and rinsing with ultrapure water. Immediately following collection of a 24-hr urine sample, the total volume was recorded and an 8-mL subsample of urine was removed and filtered through an acid-cleaned 0.5-µm Teflon syringe filter to remove any suspended particles. Samples were filtered into a polyethylene bottle and stored frozen until analyzed. Urine sample collection blanks were collected to evaluate the possibility of sample contamination with Pb from food, feces, and the metabolic cages, as described elsewhere (*16*). Fecal samples were stored frozen in acid-cleaned polypropylene jars.

Sample processing and analyses. All sample processing and analyses were conducted at the University of California, Santa Cruz, using trace metal clean techniques under HEPA filtered-air conditions to minimize contamination of the samples (*22*). We thawed and acidified (pH < 2) daily urine samples with 16N Q-HNO$_3$ (Fisher Scientific, Pittsburgh, PA) to prevent loss of trace elements to the walls of the polyethylene storage bottles; we analyzed samples directly by inductively coupled plasma mass spectrometry (ICP-MS) (*17*). For fecal samples, we added ultrapure water to the daily sample (4 parts water:1 part feces, v/w), and subsequently homogenized the sample using a stainless steel blender. To obtain a representative sub-sample, we used a Teflon stir bar to stir the homogenate; we then transferred a subsample (> 1 g) to a Teflon vial using a large bore pipette tip. The fecal subsample

subsequently was dried at 65°C to a constant weight, digested for 8 hr in 2 mL hot 16N Q-HNO$_3$, evaporated to dryness, and redissolved in 1N Q-HNO$_3$ for ICP-MS analysis (*22,23*). Blood samples were thawed and an aliquot was mixed with 1N Q-HNO$_3$; the sample was vortexed vigorously, the formed precipitate was allowed to settle, and the supernatant was transferred and analyzed by ICP-MS. National Institute of Standards and Technology (NIST) standard reference material (SRM) 955A (Pb in blood), SRM 2670 (urine), and SRM 1577b (bovine liver) were used to evaluate procedural accuracy of the sample preparation and Pb analyses.

We measured sample Pb levels using a Finnigan MAT element magnetic sector-ICP mass spectrometer (Finnigan MAT, San Jose, CA) in multi-isotope counting mode, measuring masses of ^{201}Hg (used to correct for ^{204}Hg interference of ^{204}Pb), ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb, and ^{209}Bi, with ^{209}Bi used as an internal standard (*23*). External standardization for Pb was via the NIST SRM 981 isotopic standard and a certified Spex Pb standard (Spex Industries Incorporated, Edison, NJ) that had been independently isotopically characterized via thermal ionization mass spectrometry. Based on the SRMs, the measurement accuracy for Pb was 101% ± 5% relative SD for analyzed Pb concentrations > 0.05 ppb, with an analytical detection limit of 0.01 ppb (*23*).

Calculations and definitions. Sample Pb isotope concentrations are expressed as nanomoles Pb isotope per gram of dry weight for feces or nanomoles per milliliter for blood and urine. We calculated values for endogenous Pb accumulated from the 1-year oral Pb dosing period (i.e., inherent Pb) and for the contribution from the oral and intravenous stable Pb isotope tracers. The levels of sample total Pb reflect the sum of the inherent Pb and the administered stable Pb isotope tracers, as expressed below:

$$\text{Total Pb} = \text{Total } ^{204}\text{Pb} + \text{Total } ^{206}\text{Pb} + \text{Total } ^{207}\text{Pb} + \text{Total } ^{208}\text{Pb}, \quad (1)$$

where Total ^{204}Pb includes the contributions from inherent ^{204}Pb + iv ^{204}Pb tracer. Total ^{206}Pb includes the contributions from inherent ^{206}Pb + oral ^{206}Pb tracer.

The concentrations of stable Pb isotope tracers for each collection day were unmixed from these total values using the isotopic ratio of the inherent Pb (determined in pretracer day 0 samples):

$$^{204}\text{Pb Tracer} = \{[(^{208}\text{Pb}_{\text{Inherent}}/^{204}\text{Pb}_{\text{Inherent}}) - (^{208}\text{Pb}_{\text{day } x}/^{204}\text{Pb}_{\text{day } x})] \div (^{208}\text{Pb}_{\text{Inherent}}/^{204}\text{Pb}_{\text{Inherent}})\} \times ^{204}\text{Pb}_{\text{day } x} \quad (2)$$

$$^{206}\text{Pb Tracer} = \{[(^{208}\text{Pb}_{\text{Inherent}}/^{206}\text{Pb}_{\text{Inherent}}) - (^{208}\text{Pb}_{\text{day } x}/^{206}\text{Pb}_{\text{day } x})] \div (^{208}\text{Pb}_{\text{Inherent}}/^{206}\text{Pb}_{\text{Inherent}})\} \times ^{206}\text{Pb}_{\text{day } x} \quad (3)$$

where Inherent equals nanomoles per milliliter of Pb isotope before administration of either ^{204}Pb tracer or ^{206}Pb tracer (i.e., day 0). Day *x* equals nanomoles per milliliter of Pb isotope in the sample collected on day *x* of the succimer chelation period.

We calculated the daily total excretion of each stable Pb isotope tracer by multiplying the concentration of each Pb tracer in urine and fecal samples by the amount of urine and feces excreted on that day. The total excretion of the Pb tracers for the 5 days of the chelation period (days 1–5) were determined by summing the amounts calculated for the individual days.

The following specific outcomes were calculated to achieve the study objectives:

Endogenous fecal excretion of absorbed oral ^{206}Pb tracer $= (^{206}$Pb tracer in blood $\div ^{204}$Pb tracer in blood$) \times ^{204}$Pb tracer in feces. $\quad (4)$

(This calculation assumes that the ratio of ^{206}Pb tracer:^{204}Pb tracer excreted in the feces is identical to that in the whole blood for that collection day.)

Fecal elimination of unabsorbed oral ^{206}Pb tracer $= ^{206}$Pb tracer in feces $- $ Endogenous fecal excretion of absorbed oral ^{206}Pb tracer. $\quad (5)$

GI Absorption of oral ^{206}Pb tracer (relative percent) $= [($Oral dose ^{206}Pb tracer $- $ Fecal elimination of unabsorbed oral ^{206}Pb tracer$) \div $ Oral dose ^{206}Pb tracer$] \times 100. \quad (6)$

Body retention of oral ^{206}Pb tracer $= $ Oral dose ^{206}Pb tracer $- ($Fecal elimination of unabsorbed oral ^{206}Pb tracer $+ $ Total$_{\text{urine + feces}}$ excretion of absorbed oral $_{206}$Pb tracer$). \quad (7)$

Body retention of endogenous (iv) ^{204}Pb tracer $= $ Intravenous dose ^{204}Pb tracer $- (^{204}$Pb tracer in feces $+ ^{204}$Pb tracer in urine$). \quad (8)$

Because blood samples were collected only on days 0, 1, 3, 5, and 8 of treatment, it was necessary to interpolate blood tracer values for treatment days 2 and 4 in order to estimate the ^{206}Pb tracer:^{204}Pb tracer ratio of blood on those treatment days. This was done by fitting a first-order disappearance equation to the measured blood tracer Pb concentrations on days 0, 1, 3, and 5 for each animal and then interpolating the daily values for treatment days 2 and 4. The correlation coefficients (*r*) for the best-fit lines ranged from 0.939 to 0.994, thereby validating this approach. These interpolated values were then used in calculating the daily endogenous fecal excretion of absorbed oral ^{206}Pb tracer (Equation 4).

Statistical analyses. The daily fecal elimination of unabsorbed ^{206}Pb tracer, concentrations of blood total Pb, and BPb tracer:inherent Pb ratio data were analyzed using repeated measures analysis of variance (RM-ANOVA), where the effect of treatment (succimer or vehicle) was the between subjects factor and the effect of collection day was the within-subjects factor. All remaining data were analyzed using analysis of variance (ANOVA). We used the breeding cohort as a blocking factor in all ANOVA analyses. For RM-ANOVA, we used the Greenhouse-Geisser adjusted F statistic if the test of sphericity was significant. For significant ANOVA effects, differences among means were determined using the Tukey pairwise-comparison test. We considered *p*-values < 0.05 statistically significant for all tests. All analyses were conducted using SAS Version 6.12 (*24*).

Results

Gastrointestinal absorption of oral Pb. The daily fecal elimination of unabsorbed oral ^{206}Pb tracer was significantly increased by succimer (Figure 1A). There was no succimer × collection day interaction, implying that the effect of succimer on fecal elimination of unabsorbed Pb was consistent across treatment days 1–5. When the daily fecal Pb excretion was averaged across treatment groups, it was apparent that the majority (> 96%) of unabsorbed fecal ^{206}Pb tracer was eliminated over treatment days 1 and 2 and declined substantially to near 0 by day 5 in these juvenile monkeys (Figure 1A). These results indicate that the 5-day fecal collection period was adequate to recover essentially all of the unabsorbed oral ^{206}Pb tracer that was eliminated in the feces.

Consistent with the above results, succimer significantly decreased the GI absorption of oral ^{206}Pb tracer by approximately 43% relative to the vehicle group (Pb absorption was 64.9% ± 5.5 SEM for vehicle and 37.0% ± 5.8 SEM for succimer; Figure 1B). Data from one vehicle-group monkey was excluded from the statistical analysis as an outlier because its absorption and body retention of oral ^{206}Pb tracer and its fecal elimination of unabsorbed oral ^{206}Pb tracer were > 1.5 interquartile ranges from the 75th percentile. When the observations from this animal were included in the statistical analysis,

the numerical trends remained the same (e.g., GI Pb absorption was 56% + 10 SEM for vehicle and 37% + 6 SEM for succimer), but differences between treatments were no longer statistically significant. A possible explanation for this outlying value is that this subject inadvertently received a larger dose of oral [206]Pb tracer than was intended. This animal was not an outlier for the other outcomes measured (e.g., [204]Pb tracer measures), and its inclusion in the statistical analysis of those other measures did not affect the outcome of those analyses. Nonetheless, for consistency, we excluded all measurements from this monkey from the final statistical analysis and presentation of all data.

Urinary and endogenous fecal excretion of body Pb. Succimer significantly increased the urinary excretion of total Pb (i.e., Pb tracers + inherent non-tracer Pb), but had no measurable effect on the fecal excretion of endogenous total Pb (Figure 2A). The net result was a significant overall 189% increase in the combined (urine + feces) excretion of total Pb with succimer treatment over the vehicle group. Excretion of the [204]Pb and [206]Pb tracers (Figure 2B,C) did not have a substantial influence on the excretion of total Pb, as evidenced by the fact that the combined excretion of both tracers composed < 6% of the urinary excretion and < 4% of the fecal excretion of total Pb. This point is further substantiated by the fact that statistical analysis of inherent (i.e., non-tracer) Pb values produced the same outcome as the analyses of the total Pb values (data not shown). As stated above, inherent Pb is composed predominantly of non-tracer Pb accumulated from the chronic oral Pb exposures received until 52 weeks of age.

Notably, the urinary and fecal excretion patterns of total Pb, and iv [204]Pb and oral [206]Pb tracers were similar within each treatment group, when the excretion of each tracer is expressed relative to its internal ([204]Pb) or absorbed ([206]Pb) dose (Figure 2). For this, the internal dose of [204]Pb was equivalent to the entire administered iv dose (24.5 nmol), whereas the absorbed dose of the oral [206]Pb tracer was the calculated amount absorbed across the GI tract for each animal. The lack of a statistically significant effect of succimer on the excretion of total Pb and [206]Pb tracer (Figure 2A,B) compared to [204]Pb tracer (Figure 2C) is likely due to increased variability in the former data, rather than in a specific effect of succimer per se. Overall, the effects of succimer on [204]Pb tracer (Figure 2C) may more accurately reflect the effects of succimer on endogenous body Pb because all animals received the same dose and the [204]Pb tracer was administered directly into the circulation.

The qualitative similarity in effects of succimer treatment on the excretion of the oral [206]Pb and iv [204]Pb tracers substantiates the use of this stable isotope tracer method for the assessment of the effects of succimer on GI Pb absorption and body Pb retention. These data also indicate that once in the body all sources of excretable Pb behaved similarly, regardless of whether the Pb originated from the oral ([206]Pb tracer), iv ([204]Pb tracer), or inherent (chronic through age 52 weeks) exposures.

Body retention of Pb. Succimer reduced the body retention of endogenous Pb by approximately 10% compared to the vehicle group, based on assessment of both the orally administered [206]Pb and iv administered [204]Pb tracers, although only the effect on [204]Pb tracer was statistically significant (Figure 3). This is likely due to the confounding effects of interanimal differences in the GI absorption of [206]Pb tracer. It is noteworthy that the apparent benefits of succimer treatment are substantially greater when we consider its efficacy for both reducing GI Pb absorption and increasing excretion of body Pb relative to the vehicle treatment. This greater overall benefit of succimer treatment is substantiated by the observation that the dose of oral [206]Pb tracer remaining in the body at the end of the collection period was decreased significantly by 44% versus the vehicle group, which considers losses from both fecal elimination of unabsorbed Pb and total excretion (urine + endogenous fecal) of absorbed Pb.

BPb concentrations over treatment. Succimer significantly reduced whole BPb
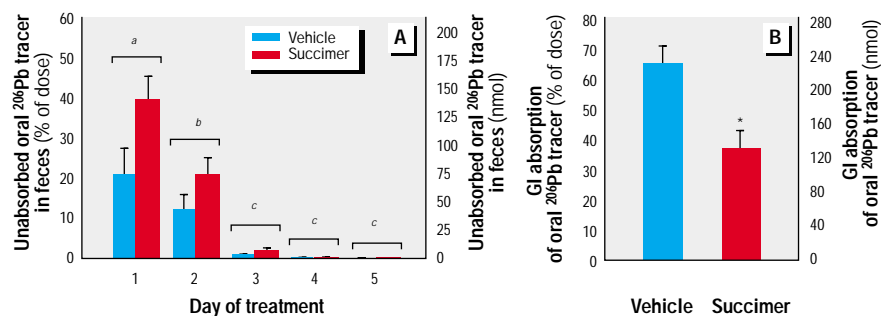
Figure 1. Effect of oral succimer on (*A*) the daily fecal elimination of unabsorbed oral [206]Pb tracer and (*B*) the GI absorption of oral [206]Pb tracer (see "Materials and Methods" for details). The bars represent mean ± SEM for the vehicle (*n* = 7) and succimer (*n* = 9) groups. Succimer increased oral [206]Pb tracer elimination in the feces according to a RM-ANOVA ($p < 0.05$). Day of treatment means with different superscripts differ according to Tukey's test ($p < 0.05$).
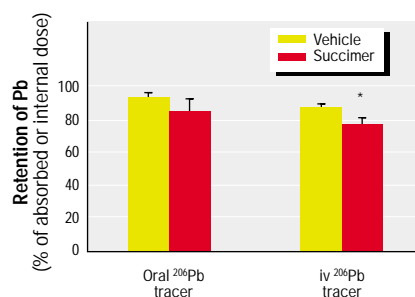*Succimer group differs from the vehicle group according to ANOVA ($p < 0.05$).
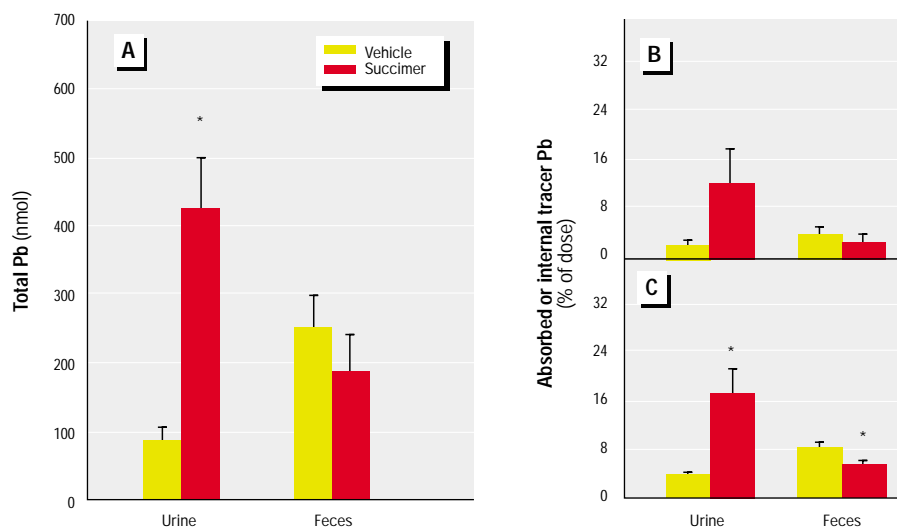
Figure 2. Effect of oral succimer on (*A*) urinary excretion and endogenous fecal excretion of total Pb (inherent Pb + Pb isotope tracers); (*B*) urinary and fecal excretion of oral [206]Pb tracer over the first 5 days of treatment; and (*C*) urinary and fecal excretion of endogenous [204]Pb tracer over the first 5 days of treatment. The bars represent mean ± SEM for the vehicle (*n* = 7) and succimer (*n* = 8–9) groups. In (*B*), succimer did not measurably affect urinary or fecal excretion of the oral [206]Pb tracer when the tracer is expressed as a percentage of the amount absorbed from the GI tract. In (*C*), succimer increased urinary excretion and decreased fecal excretion of endogenous [204]Pb tracer (ANOVA, $p < 0.05$), when tracer is expressed as a percentage of the amount injected iv.
*Succimer increased urinary excretion of total Pb versus vehicle according to ANOVA ($p < 0.05$).

concentrations measured on days 1, 3, 5, and 8 of the treatment period, when these values were normalized to initial concentrations (i.e., percentage of day 0 values for each animal) (Figure 4). When analyses were conducted using the absolute (nonnormalized) data, succimer treatment was found to significantly reduce BPb levels only on days 3 and 5 (Figure 4B). This latter outcome is attributed to interanimal variability in initial BPb levels within each treatment group, which was factored out when the data were normalized to each animal's day 0 BPb value. Notably, BPb levels in the vehicle-treated group did not measurably decline over the 8 days of treatment (Figure 4), which is attributable to the fact that Pb exposure ceased immediately before the first chelation regimen 12 weeks earlier. It is also noteworthy that the BPb levels in these animals were not increased by the administered stable Pb isotope tracers. For instance, the [204]Pb tracer was on average only 0.82% ± 0.18 SEM of the total BPb for the vehicle group and only 1.0% ± 0.17 for the succimer group, whereas the [206]Pb tracer was 3.1% ± 0.92 and 1.3% ± 0.35 of total BPb for the vehicle and succimer groups, respectively.

Succimer did not affect the ratio of [204]Pb tracer:inherent Pb in the blood because that ratio was not measurably different between the vehicle and succimer groups on any treatment day (data not shown). This outcome further substantiates that endogenous body Pb responded similarly to succimer, regardless of its source of exposure (i.e., inherent Pb, iv administered [204]Pb tracer). This was also apparent from the results on the excretion of endogenous Pb presented above (urinary and endogenous fecal excretion of body Pb). In contrast, succimer significantly decreased the ratio of oral [206]Pb tracer:inherent Pb in the blood by approximately 70%, and this decrease persisted throughout treatment days 1–8 (data not shown). This outcome may be simply attributed to the effect of succimer on reducing the absorption of the oral [206]Pb tracer, which resulted in a reduced appearance of [206]Pb tracer in the blood and hence a lower [206]Pb tracer:inherent Pb ratio in blood.
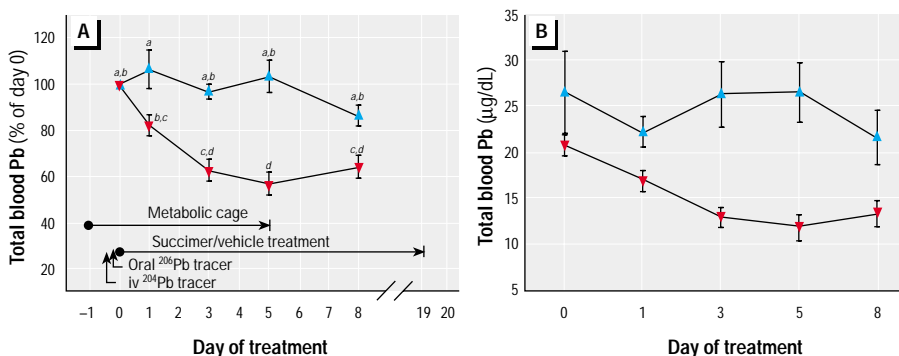
Discussion

Succimer decreased gastrointestinal absorption of oral Pb. We observed that oral succimer treatment significantly decreased (by 43%) the GI absorption of Pb that was present in the GI tract concurrent with beginning treatment. This effect is demonstrated not only by the increased fecal recovery of unabsorbed [206]Pb tracer in the succimer group (Figure 1A) but also by the decreased appearance of oral [206]Pb tracer in the circulation compared to the vehicle controls. The latter is evidenced by the ratio of [206]Pb tracer:inherent Pb in blood, which was reduced by approximately 70% with succimer treatment (data not shown). Moreover, it is clear that this latter result is not due to a different effect of succimer on inherent versus tracer Pb within the body (e.g., Pb in the circulation) per se because succimer treatment had no measurable effect on the ratio of iv [204]Pb tracer:inherent Pb.

These results substantially extend previous studies that have investigated the effects of succimer on GI Pb absorption. In a study in rodents, Kapoor et al. (10) administered a single radioactive Pb tracer dose followed by a single dose of DMSA (~ 20 mg/kg) and found that oral succimer decreased GI Pb absorption, although succimer administered via intraperitoneal injection caused an opposite increase in GI Pb absorption. A more recent study in non-Pb exposed adult humans used a stable Pb isotope tracer (200 µg [204]Pb administered orally) method and a single dose of oral succimer (0, 10, or 30 mg/kg) and found that succimer had no measurable effect on GI Pb absorption (8). However, trends in those data suggested that GI Pb absorption was enhanced by succimer. There are several reasons, including a possible age effect, that may account for these study differences. The adult human study used a lower oral Pb tracer dose (~ 2.5 µg or 12 nmol [204]Pb/kg body weight), a single oral dose of succimer, and a shorter study duration after succimer and Pb dosing (~ 26 hr), the latter of which may not have been sufficient for complete fecal collection of unabsorbed Pb tracer. By comparison, in the present study we used a higher oral Pb tracer dose of approximately 25 µg or 121 nmol [206]Pb/kg body weight, a succimer regimen of three divided doses per day for multiple days, and complete urine and fecal collections over the first 5 days of treatment.

Succimer significantly increased the excretion of Pb in urine. The effect of succimer treatment to significantly increase the excretion of Pb in the urine is consistent with the action of this drug to form aqueous soluble chelates with Pb (12), as well as with a large body of clinical and laboratory evidence showing increased urinary excretion of Pb with treatment (1,17,25,26). This effect was similar for both the iv-administered [204]Pb tracer and the oral [206]Pb tracer that had been absorbed, and for the urinary excretion of inherent body Pb originating from the chronic oral Pb exposures (Figure 2). Although not apparent from these data, other stable Pb isotope tracer data from the larger cohort of monkeys (n = 48) of the parent study indicate that succimer chelates and removes Pb from labile soft tissue compartments and not from the skeleton (17).

Succimer tended to reduce the excretion of endogenous Pb via the fecal route

Figure 3. Effects of oral succimer on retention of absorbed GI (oral [206]Pb tracer) and endogenous (iv [204]Pb tracer) Pb expressed as a percentage of the internal dose. The internal dose is the amount of the endogenous [204]Pb tracer injected iv or the amount of the oral [206]Pb tracer absorbed from the GI tract. The sum of the tracer Pb retained over the first 5 days of treatment is expressed as a percentage of the internal dose, which equals the amount of oral [206]Pb tracer absorbed or the amount of [204]Pb tracer that was injected. The bars represent mean ± SEM for the vehicle (n = 7) and succimer (n = 9) groups.

*Succimer group differs from the vehicle group according to an ANOVA (p < 0.05).

Figure 4. Effects of oral succimer on total BPb (inherent Pb + Pb isotope tracers) concentrations (A) expressed as a percentage of the initial day 0 value within each animal and (B) expressed as absolute total BPb levels (µg/dL) for the vehicle (blue; n = 7) and succimer (red; n = 9) groups. The day 0 sample was collected before the tracers and succimer were administered. Means with different superscripts differ according to Tukey's test (p < 0.05). In (B), the succimer group differs from the vehicle group only on treatment days 3 and 5 according to Tukey's test (p < 0.05; superscripts were omitted for clarity).

compared to the vehicle treatment (Figure 2). Although this effect reached statistical significance only for the ^{204}Pb tracer, data for the fecal excretion of ^{206}Pb tracer and endogenous total Pb exhibited similar trends. Because this reduction in fecal Pb excretion with succimer treatment was small compared to the increase in urinary excretion, the overall elimination of body Pb was still substantially increased with succimer treatment.

Succimer decreased the retention of Pb in the body. The effect of succimer to decrease the net body retention of Pb is consistent with its effect on increasing urinary Pb excretion, relative to the vehicle group (Figure 2). A reduction in body Pb retention was evident with the recovery of both oral ^{206}Pb and iv ^{204}Pb tracers, again indicating that succimer acts equally on Pb from past chronic exposures and recent oral exposures concurrent with treatment (Figure 3). This outcome is consistent with several previous rodent studies which showed that oral succimer decreased the whole-body retention (*10*) and tissue accumulation (*27*) of Pb derived from concurrent oral Pb exposures. These data are in contrast with a previous rodent study of several other chelating agents, including BAL, citrate, and penicillamine, which suggested that these agents may increase the body absorption and retention of Pb from exposures suffered concurrent with treatment (*11*).

Although succimer significantly decreased the whole-body retention of Pb, the large majority of the administered ^{204}Pb and absorbed oral ^{206}Pb tracers were retained in the body (> 77%; Figure 3), despite treatment with succimer. This result is not due to the incomplete collection of excreted Pb tracers because most (e.g., > 80%) of the total 5-day urinary and endogenous fecal excretion of both tracers occurred over the first 3 days of treatment (daily endogenous fecal and urine data not shown). This is surprising because the majority of absorbed succimer remains in the extracellular compartment (*28,29*) and binds Pb with high affinity (*12*). Nevertheless, this outcome is supported by a previous study in rodents, which suggested that succimer treatment increased the enrichment of GI-absorbed Pb into the skeleton while also causing increased urinary excretion of Pb (*30*).

Gastrointestinal absorption and excretion of Pb in the absence of succimer therapy. The relative absorption (~ 65%, fasting conditions), and subsequent excretion (3.9%) and retention (61%) of orally dosed ^{206}Pb tracer in the vehicle group are in general agreement with the ranges observed in previous studies in monkeys (*31,32*). Further, we observed that the excretion of absorbed Pb via urinary and endogenous fecal routes were comparable in magnitude.

These latter results are consistent with studies in humans (*33*), monkeys (*31*), and rodents (*34–36*), and collectively demonstrate that endogenous fecal excretion is significant as a route of Pb elimination from the body.

Effects of succimer on BPb level. The significant reductions in total BPb levels over the first 8 days of succimer treatment are consistent with the larger data set containing these animals (*n* = 22–24/treatment) that showed significant reductions in BPb level over both the first and second chelation regimens (*17*). These data are also consistent with studies in adult monkeys (*37*) and studies in children (*1,25*). Our observation that total BPb levels in the vehicle group did not measurably decline over the study period may simply be attributed to the fact that oral Pb exposure to these animals had ceased at 52 weeks of age, 12 weeks before the present study. As such, the reduction in BPb level with cessation of exposure had already reached an asymptotic function of slow decline when this study was conducted.

Clinical relevance. The succimer placebo-controlled and double-stable Pb isotope tracer design of the present study was ideally suited to definitively address the effects of succimer on the GI absorption and body retention of Pb. The juvenile monkey model used here closely approximates human metabolism for both Pb (*17,38*) and succimer (*12,39,40*), and thus is the most appropriate model available for studies on the effects of succimer on the disposition of GI Pb in children. Adult primates, including humans, are not well suited for estimating GI Pb absorption in juveniles because of the confounding effects of age (*32,41,42*). Moreover, existing data suggest that there may be species differences (rodents vs. primates) in the pharmacokinetics of succimer (*12,39*), suggesting that rodent studies on some effects of succimer may not directly extrapolate to humans. Finally, the Pb exposure levels and succimer treatment regimens are comparable to those experienced by Pb-poisoned children, and the administered oral ^{206}Pb tracer dose is well within the range of oral daily exposure suffered by Pb-exposed children (~ 116 μg/day or 562 nmol/day) (*43*).

The ability of succimer to reduce GI Pb absorption has important implications for the clinical treatment of Pb-poisoned children. Our results suggest that children who continue to be exposed to Pb during succimer chelation are not likely to be at risk of increased GI Pb uptake due to succimer treatment. However, this does not reduce the importance of primary prevention of Pb exposures to children, nor does it support the use of succimer as a prophylactic for Pb exposure, because substantial amounts of Pb from the GI tract were absorbed and retained

in the body despite the reductions in GI Pb absorption caused by succimer.

Although the animal model used in this study is highly relevant to childhood Pb intoxication, care should be taken in extrapolating the results from this study to children because the experimental conditions used here do not cover the broad range of conditions that could be encountered clinically. For instance, these animals received optimal nutrition, a continuous steady exposure to Pb early in life, a fasting period before administration of the oral ^{206}Pb tracer and first succimer dose, and only a single oral dose of Pb (tracer) immediately (within 15 min) before beginning succimer treatment. It is likely that the nutritional and Pb exposure history of these monkeys differed from that experienced by children suffering from elevated Pb exposure. Thus, although the present study indicates that succimer does not increase GI Pb absorption, we cannot definitively conclude that succimer can safely be given to children while they have ongoing Pb exposures. Finally, the outcomes reported here should not be extrapolated to succimer treatment regimens in which succimer is administered by a different route because previous studies in rodents have shown that parenteral administration of succimer increased the GI absorption of Pb (*10*).

Summary

These results support the hypotheses that oral succimer therapy reduces the absorption of Pb from the GI tract and subsequently reduces the body retention of Pb. These outcomes have important clinical implications for the out-patient treatment of Pb-poisoned children with succimer because Pb exposure may continue during treatment in some of those children due to the scarcity of Pb-safe housing and uncertainties in identifying and remediating environmental Pb exposures.

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