

Reductions in Blood Lead Overestimate Reductions in Brain Lead after Repeated Succimer Regimens in a Rodent Model of Childhood Lead Exposure

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Although many studies have demonstrated the efficacy of succimer chelation in reducing blood and brain lead levels, the relative efficacy of the drug in the two tissues is less well understood. This issue is important because blood lead levels after chelation are used clinically to estimate reductions in the brain, the most critical organ in considering lead-induced neurotoxicity. The present study was designed to further investigate this issue, using multiple chelation regimens. Long-Evans rats were exposed to one of three lead exposure regimens from birth until postnatal day 40, followed by treatment with succimer (one or two 3-week regimens) or vehicle. The results indicated that one succimer regimen was significantly superior to vehicle treatment in lowering lead levels in both blood and brain across the entire 8-week follow-up period. Similarly, a second succimer regimen offered significant additional benefit relative to one regimen for both blood and brain across the 4-week follow-up period. However, several findings revealed that succimer-induced reductions in brain lead lagged behind reductions in blood lead and were generally smaller in magnitude. Furthermore, a rebound was detected in blood, but not brain, lead levels after both succimer regimens. Given the results of this study, we urge caution in using blood lead as a surrogate for brain lead levels, particularly during and immediately after chelation treatment when reductions in blood lead levels overestimate reductions in brain lead levels. The present results suggest that, in clinical use, succimer treatment may need to extend beyond the point at which blood lead levels have dropped to an "acceptable" target value in order to effectively reduce brain lead levels and minimize neurotoxicity. **Key words:** chelation, dimercaptosuccinic acid, lead exposure, lead poisoning, neurotoxicity, succimer. *Environ Health Perspect* 112:302–308 (2004). doi:10.1289/ehp.6517 available via <http://dx.doi.org/> [Online 31 October 2003]

Epidemiologic studies over the past several decades have suggested that even slightly elevated lead levels during early childhood can produce cognitive impairments and behavioral problems that endure long past the period of exposure (e.g., Bellinger et al. 1984, 1992; Dietrich et al. 1993; Fergusson et al. 1993; Needleman et al. 1990). In fact, Canfield et al. (2003) recently reported a significant inverse correlation between IQ and blood lead levels across the range of 2–10 µg/dL, suggesting adverse effects at lead levels lower than previously recognized. Importantly, studies using animal models have also demonstrated lasting cognitive and affective changes in animals exposed to lead early in life (e.g., Garavan et al. 2000; Levin and Bowman 1986; Morgan et al. 2001; Rice 1992; Stangle et al. 2003). These latter findings provide important evidence that early lead exposure can, in fact, cause these lasting deficits, an inference that has remained tentative in human studies because of the correlational nature of the data and frequent confounding of lead exposure and other risk factors for impaired cognitive and affective development.

Several studies have shown that interventions focusing on abatement and education produce only moderate reductions in lead levels over prolonged periods of follow-up. For example, Roberts et al. (2001) showed that blood lead levels of 25–29 µg/dL required

24 months to decline to < 10 µg/dL (the current level of concern for children), although all families received education, counseling, lead hazard identification, and relocation when possible. These data underscore the need for therapeutic chelating agents to reduce body lead levels more rapidly than can be achieved through environmental efforts alone.

Therapeutic chelation treatment has been used for decades to treat childhood lead poisoning (for reviews, see Graziano 1986; Graziano et al. 1985). According to current Centers for Disease Control and Prevention (CDC) guidelines (2002), chelation with Versenate (CaNa₂EDTA) is indicated for children with blood lead levels ≥ 45 µg/dL, but more ambiguity exists regarding optimal therapeutic intervention for children with moderately elevated lead levels (25–45 µg/dL). The use of the oral chelating agent succimer has increased over the last several years because of its benefits over Versenate. Succimer is orally effective and is more specific for lead than is Versenate, thereby avoiding the diuresis of essential elements (e.g., calcium and zinc; Bentur et al. 1987; Chisolm 2000; Flora et al. 1995; Graziano et al. 1988; Kostial et al. 1999; Smith et al. 2000a; Thomas and Chisolm 1986).

Most studies demonstrating the effectiveness of succimer (and Versenate) in reducing tissue lead levels have assessed efficacy soon

after the completion of treatment. However, recent clinical and nonhuman primate studies have suggested that succimer may be no more effective in reducing body lead levels than the simple cessation of lead exposure, when treatment efficacy is evaluated over longer periods of time after chelation (Cremin et al. 1999; Liu et al. 2002; O'Connor and Rich 1999; Rogan et al. 2001; Ruff et al. 1993; Smith et al. 2000b). Moreover, the one clinical trial of succimer treatment that has included cognitive outcomes [Treatment of Lead-Exposed Children (TLC) Trial Group 2000] was unable to detect a benefit of succimer treatment (relative to placebo) on cognitive functioning (Rogan et al. 2001). It is possible that the duration of succimer treatment in the TLC study was insufficient to significantly lower brain lead levels, despite the lowering of blood lead levels. This possibility is based on the evidence from animal studies that succimer-induced reductions in brain lead lag behind reductions in blood lead (Cremin et al. 1999; Smith et al. 1998), and that extended succimer treatment can continue to lower brain lead levels without continued reductions in blood lead (Smith et al. 1998).

In clinical practice, blood lead levels are used to determine the duration of chelation therapy (i.e., number of regimens) and assess its efficacy. Yet, as noted above, recent animal studies indicate that chelation-induced reductions in brain lead significantly lag behind changes in blood lead. As a result, in the short term, changes in blood lead may greatly overestimate the efficacy of chelation in reducing brain lead (Cremin et al. 1999; Smith et al. 1998). It follows that extending the duration of succimer treatment will significantly increase the efficacy of the drug in removing brain lead and, ideally, lessen the resulting cognitive dysfunction. For these reasons, there is a pressing need to determine the efficacy of varying durations of succimer treatment on blood and brain lead levels, as well as the more general issue of the correspondence between

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blood and brain lead levels, particularly after chelation therapy.

In the present study we used a rodent model of childhood lead exposure to systematically investigate *a*) the efficacy of single versus repeated succimer treatment regimens for reducing blood and brain levels, *b*) the extent to which blood lead can serve as a proxy for brain lead during and after chelation, and *c*) whether rebounds in blood and brain lead levels occur after chelation therapy, in the absence of environmental reexposure.

Materials and Methods

Experimental design. The present study was a 3×3 factorial design, involving three levels of lead exposure and three levels of chelation therapy (vehicle, one 3-week succimer regimen, two 3-week succimer regimens). Animals were followed for a total of 11 weeks after cessation of lead exposure, corresponding to 8 weeks after the first chelation regimen ended and 4 weeks after the second regimen ended. As depicted in Figure 1, animals were sacrificed at various time points throughout the study to assess the efficacy of succimer in reducing blood and brain lead levels. This lengthy follow-up period allowed assessment of succimer efficacy in both blood and brain across various postchelation time periods, as well as an assessment of the potential “rebounding” of blood and brain lead levels after cessation of chelation.

Animals. Nulliparous Long-Evans rats (Harlan Sprague Dawley, Indianapolis, IN) were housed in animal facilities accredited by the Association for the Assessment and Accreditation for Laboratory Animal Care, with a 12:12-hr light:dark cycle. Food (Pro-Lab RMH 1000; Nutrition International LLC, Brentwood, MO) and water were available *ad libitum*. Rats were mated at approximately 10 weeks of age. Pregnancy was determined by the presence of a sperm plug and subsequent weighing of the females at approximately 5-day intervals. Dams were maintained on tap water and laboratory chow during gestation.

Lead exposure. Lead was administered from birth until postnatal day (PND) 40. Until weaning on PND21, the pups received lead via nursing from lead-exposed dams, whereas from PND21 through PND40, the pups directly consumed lead from their water supply. At birth, each dam was randomly assigned to a lead exposure condition. All dams received lead via drinking water (600 $\mu\text{g}/\text{mL}$ lead as lead acetate) during PND1–18, followed by daily oral administration of an equivalent lead dose to the dams via eye dropper until weaning on PND21. After weaning, the lead exposure diverged into two groups as follows: *a*) 20 $\mu\text{g}/\text{mL}$ lead in the drinking water during PND21–28, followed by 30 $\mu\text{g}/\text{mL}$

during PND29–40, or *b*) 40 $\mu\text{g}/\text{mL}$ lead in the drinking water during PND21–28, followed by 60 $\mu\text{g}/\text{mL}$ during PND29–40. Lead exposure in all animals was terminated at PND40.

The decision to give all dams the same lead dose during lactation (PND1–21) was based on prior studies in our laboratory showing that pup blood lead levels remain relatively low and constant across a wide range of maternal intakes (15–25 $\mu\text{g}/\text{dL}$) (Garavan et al. 2000; Morgan et al. 2001) and do not increase until very large doses are used that produce maternal toxicity (unpublished data). The selection of lead doses after weaning was also based on prior studies in our lab (unpublished data). The lower-dose regimen produces relatively consistent, low blood lead levels, whereas the higher-dose regimen produces a wider range of blood lead levels. These two doses together therefore allowed us to examine low, moderate, and high exposure in the pups.

Twenty-four hours after birth, the litters were culled to 10 pups. Within each lead treatment condition, 10 subgroups were defined by the five sacrifice dates and three succimer treatment conditions (vehicle, one succimer regimen, two succimer regimens; see Figure 1). When possible, one pup per litter was assigned to each of these 10 subgroups, with the goal of providing littermate comparisons for these 10 conditions, and to avoid having more than one animal per litter in a given subgroup.

Chelation. Succimer (Chemet; Sanofi-Synthelabo Inc., New York, NY) was administered via oral gavage at a dose of 50 mg/kg/day for 1 week followed by 25 mg/kg/day for an additional 2 weeks, for a total treatment duration of 3 weeks per regimen. Succimer was dissolved in apple juice and administered within 15 min of mixing. The vehicle groups received equivolume apple juice carrier, also by gavage.

The daily dose of succimer or vehicle was divided and administered as two equal doses given 10–12 hr apart.

Sample collection and analysis. Animals were sacrificed at five time points throughout the study (Figure 1) to assess the efficacy of the various treatments in lowering blood and brain lead levels, as follows: *a*) One animal per litter was sacrificed at PND41, immediately after cessation of lead exposure, and immediately before the start of chelation. Sacrifice at this time point provided a representative measure of the tissue lead levels for each litter. *b*) On PND62, immediately after the first round of chelation treatment, both succimer- and vehicle-treated animals from each lead exposure condition were sacrificed to assess the immediate efficacy of one chelation regimen. *c*) An additional group of rats that received one round of succimer treatment were sacrificed at PND69, 1 week after treatment ended and immediately before the second round of chelation treatment began, to examine whether a rebound in blood and/or brain lead levels occurred after cessation of succimer treatment. *d*) Rats from each chelation treatment group (vehicle, one succimer regimen, two succimer regimens) were sacrificed at PND90, immediately after the second round of chelation (and 4 weeks after the first round of chelation ended). Sacrifice at this time served a dual purpose to examine the long-term effects of one round of succimer treatment and the added benefit of a second round of succimer treatment compared with one round of treatment. *e*) Finally, animals from each of the three chelation conditions were sacrificed at PND118, 8 weeks after the first chelation regimen and 4 weeks after the second regimen. Sacrifice at this time point also served a dual function: to assess the long-term efficacy of one and two succimer regimens relative to vehicle

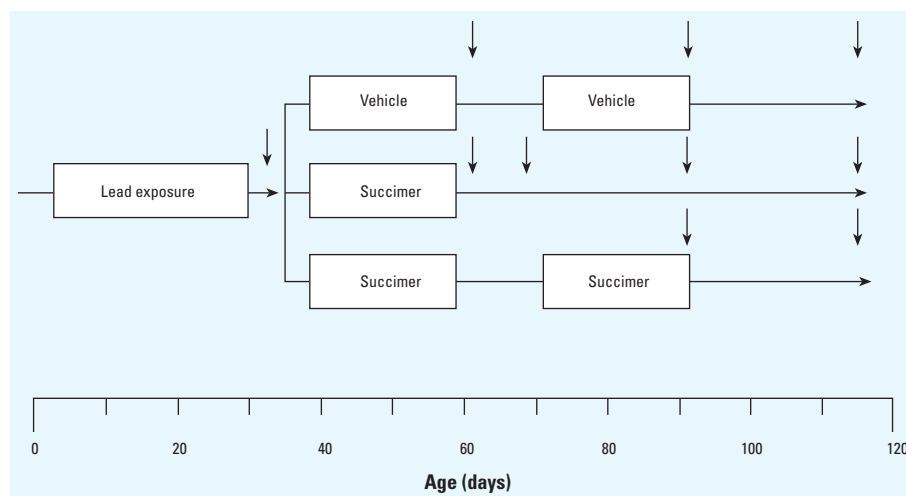


Figure 1. Study design. Lead exposure occurred from PND0 through PND40, followed by either one 3-week regimen of succimer, two 3-week regimens of succimer, or vehicle treatment. Arrows represent days at which rats from each lead exposure group were sacrificed and blood and brain lead levels analyzed.

treatment and relative to each other, and whether a rebounding occurred in blood or brain levels after the second chelation regimen.

At the time of sacrifice, a 2- to 3-mL sample of whole blood was collected into a polypropylene syringe via cardiac puncture from surgically exposed hearts of anesthetized animals and deposited into Vacutainers specified for trace metal (no. 367734, Becton Dickson, Research Triangle Park, NC). Animals were given a sodium pentobarbital overdose (50 mg/kg) and exsanguinated. Whole brain was removed using acid-washed stainless-steel dissecting tools, rinsed with Milli-Q water, and deposited into polypropylene storage containers. All tissue sampling was conducted using trace-metal-clean procedures. Dissecting instruments (stainless steel) were cleaned before each dissection and rinsed frequently within a dissection procedure to avoid contamination from nonbrain tissues. All samples were stored frozen.

Lead concentrations were measured in whole blood and brain tissue. Blood lead levels were determined using graphite furnace atomic absorption spectroscopy at the Wisconsin State Laboratory of Hygiene (WSLH; Madison, WI). The WSLH administers the Nationwide Blood Lead Proficiency Testing Program in cooperation with the Centers for Disease Control and Prevention and the Maternal and Child Health Bureau. Brain lead levels were measured at the University of California, Santa Cruz, using a Finnegan-element inductively coupled plasma (ICP)/high-resolution mass spectrometer (MS) in multi-isotope counting mode, measuring masses ^{208}Pb and ^{209}Bi , with ^{209}Bi used as an internal standard (Smith et al. 2000a, 2000b; Woolard et al. 1998). External standardization was via a certified lead standard that had been isotopically characterized independently via thermal ionization MS. National Institute of Standards and Technology (Gaithersburg, MD) Standard Reference Materials 955A (blood) and 1577 (bovine liver) were used to evaluate procedural accuracy. This ICP/MS methodology has been demonstrated to yield a measurement precision of $< \pm 0.5\%$ for sample lead concentrations of ≥ 0.05 ppb (Woolard et al. 1998). The analytic detection limit was 0.01 ppb.

Statistical analysis. All statistical analyses were conducted using SAS 8.2 (SAS Institute, Inc., Cary, NC). The present data contained correlated observations due to the use of two observations from each animal (blood and brain) and due to the use of multiple animals per litter. Therefore, a repeated-measures analysis of variance model (PROC MIXED; Littell et al. 1996) was used. To analyze the data for both tissues in a single analysis, blood and brain lead concentration data were first converted to comparable units of parts per billion (i.e., nanograms per milliliter and nanograms

per gram, respectively). However, box plots indicated that the scale and skewness were larger in the brain samples than in the blood samples. A natural log transformation was applied to normalize the residuals and reduce the scale of the random effects. Taking a log transformation is often successful when the effects in the model are proportional rather than additive.

The estimated least-squares means produced in the analysis of log-transformed data are expressed as geometric means after back-transforming to the original units. Exponentiation of the difference in least-squares means on the analysis scale is equivalent to the ratio of geometric means on the original scale. Therefore, the usual tests of effects involving differences in means on the analysis scale (i.e., succimer minus vehicle) are actually assessing proportional, or relative, changes in the original units (i.e., succimer/vehicle). Figures are presented in the original scale for ease of interpretation. Standard errors for means are not included on the figures because the appropriate standard error of a difference in means cannot be derived directly from the individual mean standard errors because of the covariance terms, which vary by the particular groups being compared.

The “one succimer regimen” analysis investigated vehicle versus one cycle of succimer treatment at three time points: PNDs 62, 90, and 118. The “two succimer regimens” analysis investigated the efficacy of vehicle, one cycle of succimer, and two cycles of succimer at the two time points after the second chelation regimen: PNDs 90 and 118. The initial models were constructed containing the main effects of tissue type (blood or brain), lead exposure group, succimer treatment (vehicle, one cycle, or two cycles), and time (age at sacrifice). Preliminary analysis of the animals sacrificed immediately after cessation of lead exposure (PND41), before chelation, confirmed that the higher exposure regimen produced a much wider range of blood lead levels (range, 25–160 $\mu\text{g}/\text{dL}$) than did the lower exposure regimen (range, 20–30 $\mu\text{g}/\text{dL}$). Therefore, for the two analyses of the chelation treatment effects, a three-level lead exposure variable was created, in which each animal’s lead exposure designation was based on the blood lead level of the littermate that was sacrificed at PND41. This three-level designation explained much more of the variation in the data than did a two-level designation, based on the Akaike Information Criterion (Akaike 1974; Bozdogan 1987; Wolfinger 1993). The initial models also included all relevant two-, three-, and four-way interactions involving the main effects. Each model was reduced by removing the least significant higher-order term first, reevaluating the model, and repeating the process

until all effects were either significant at approximately the 5% level or included in a higher-order term that was significant.

Results

Data. Blood and brain levels were collected from 140 animals, from 25 litters. However, only data from a slightly smaller number of samples (136 blood samples and 131 brain samples) were available for statistical analyses because of collection and/or analytic problems. In addition, in the course of statistical analysis, it was discovered that five animals had unusually high lead levels in either blood ($n = 1$) or brain ($n = 4$), without correspondingly high values in the other sample from the animal. These values were orders of magnitude different from other animals receiving the same initial exposure, treatment, and follow-up time, suggesting an analytic problem. One possible explanation for the four high brain measurements is that the samples were contaminated during collection and/or processing for analysis, possibly by the inclusion of a small skull fragment. These five observations were excluded from the analyses presented below.

As noted above, three lead groups were designated for analysis based on the blood lead level of the littermate sacrificed on PND41. The resulting low, medium, and high lead exposure groups had average (\pm SD) blood lead levels of 24.4 (\pm 3.2), 49.5 (\pm 10.8), and 131.3 (\pm 26.0) $\mu\text{g}/\text{dL}$, respectively. Average (\pm SD) brain lead levels for the three groups were 1,825 (\pm 374), 2,867 (\pm 247), and 6,781 (\pm 1,863) ng/g dry weight, respectively.

Efficacy of a single succimer treatment regimen. For each of the three time points examined (immediately after the first chelation regimen at PND62, as well as PND90 and PND118), the animals treated with succimer had significantly lower lead levels in both blood and brain than did their vehicle-treated counterparts (all $p < 0.0001$). However, as indicated by a significant interaction among treatment, tissue, and time [$F(2,156) = 6.68$, $p = 0.002$], treatment efficacy varied as a function of both tissue type and time after chelation (Figure 2). At the end of the first chelation (PND62), the reduction in brain lead produced by succimer treatment (compared with the vehicle-treated animals, i.e., succimer/vehicle) was significantly smaller than that seen in blood ($p = 0.0006$). Across the following 8 weeks of the study, brain lead continued to drop in both the succimer- and vehicle-treated animals, with the relative difference between the two groups remaining relatively constant. In contrast, blood lead levels in the succimer-treated group increased significantly (i.e., rebounded) over the 4 weeks after chelation, whereas blood lead levels in the vehicle group continued to decrease. Between PND90 and PND118 (4 and 8 weeks after chelation),

blood lead remained relatively constant in the succimer-treated animals ($p = 0.9$), at a level that was significantly lower than their vehicle-treated counterparts. These findings demonstrate that although succimer treatment was highly effective in removing lead from both tissues, the reduction in brain lead levels lagged significantly behind the reduction in blood lead levels.

A significant interaction was found between treatment and lead exposure group [$F(2, 159) = 5.65$, $p = 0.004$]. Although succimer treatment was significantly more effective than vehicle in removing lead from all three lead exposure groups ($p < 0.0001$), the magnitude of the succimer effect relative to vehicle varied by lead exposure group (Figure 3). The succimer effect relative to vehicle was largest in the low lead group and decreased with increasing lead exposure. However, as shown in Figure 3, the absolute amount of lead removed by succimer was larger in the high-lead group than in the two lower groups.

Rebound analysis after one cycle of succimer. The “rebound” analysis included data from the day immediately after chelation (PND62), as well as 1 and 4 weeks later (PND69 and PND90). There was a significant tissue type by time interaction ($p < 0.0001$). Averaged over lead exposure groups, blood lead levels exhibited a rebound 1 week after the cessation of treatment ($p < 0.0001$), but brain lead levels did not ($p = 0.80$; Figure 4). Further, whereas both blood and brain showed a decline in average lead levels from PND69 to PND90 (1–4 weeks after chelation), blood lead values at 4 weeks after chelation were still significantly higher than those seen immediately after chelation ended, as presented above. Thus, a rebound in lead levels was detected in blood but not in brain.

Analysis assessing the added benefit of a second cycle of succimer treatment. This analysis included data from PND90 and PND118, corresponding to the day immediately after the second chelation regimen ended and 4 weeks later. The analysis was performed on 88 brain specimens and 97 blood specimens, contributed by 101 animals. As depicted in Figure 5, the animals that received two cycles of succimer had significantly less lead in both brain and blood than did animals that received either one succimer regimen or vehicle treatment (all $p < 0.008$). Therefore, two cycles of succimer offered a significant added benefit relative to one cycle throughout the time period examined.

However, treatment efficacy varied as a function of tissue type, time, and lead exposure, as indicated by two borderline three-way interactions involving treatment, as well as several significant underlying two-way interactions. A borderline interaction among treatment, tissue type, and time [$F(2, 137) = 2.93$,

$p = 0.06$] reflected the fact that the magnitude of the added benefit of the second succimer regimen varied as a function of both tissue type and time since the second regimen ended. Immediately after the second chelation, the benefit of one cycle of succimer (relative to vehicle) and the added benefit of the second cycle (relative to only one cycle) were of similar magnitude in blood and brain tissue (Figure 5). However, 4 weeks later (PND118), the benefit of the second regimen (relative to vehicle) was significantly greater for brain than for blood ($p = 0.002$). This pattern of findings reflects the fact that during the final 4 weeks of the study, brain lead continued to decline in all

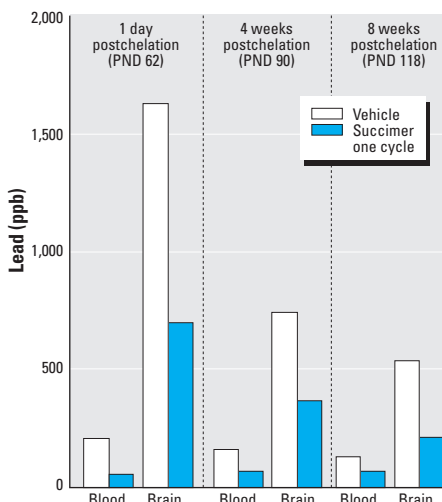


Figure 2. The efficacy of one regimen of succimer (relative to vehicle treatment) in lowering blood and brain lead levels, assessed at various times after chelation ended on PND61. Tissue lead concentrations are expressed as parts per billion (ppb); for blood and brain this is equivalent to nanograms of lead per milliliter and nanograms of lead per gram dry weight, respectively). For comparison, 10 ppb blood lead equals 1 $\mu\text{g}/\text{dL}$.

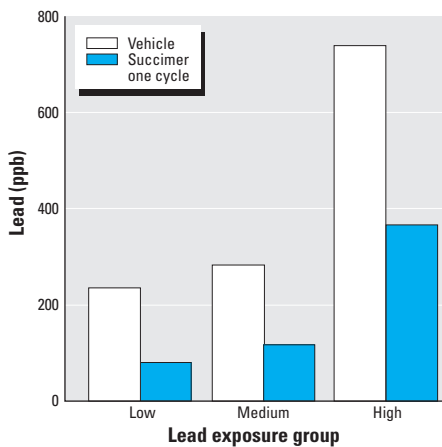


Figure 3. The efficacy of one regimen of succimer (vs. vehicle treatment) in lowering tissue lead levels (blood and brain levels combined), as a function of lead exposure, when assessed immediately after the first succimer regimen (PND62; see text for details).

treatment groups, whereas blood lead levels of the one-cycle succimer group remained relatively constant, and that of the two-cycle succimer group significantly increased (i.e., rebounded; $p = 0.05$).

A separate analysis was conducted on the blood samples from the animals receiving two cycles of succimer, based on the evidence (above) that the rebound occurred only in the blood, and because this approach decreased the residual error (the brain samples are more variable than the blood samples). This analysis showed that the average lead level in the blood was higher 4 weeks after the second chelation than immediately after cessation of the second

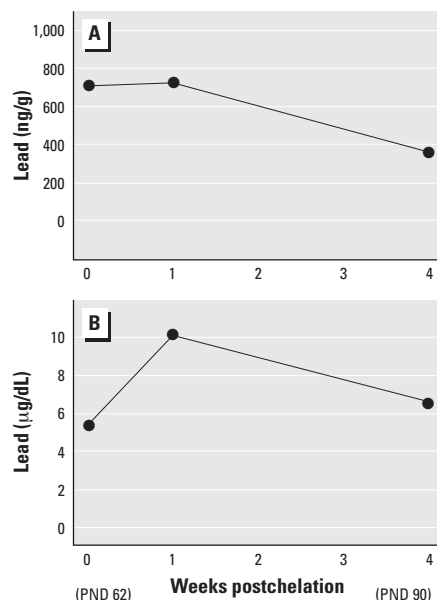


Figure 4. Levels of (A) brain and (B) blood lead across the 4 weeks after completion of the first succimer regimen.

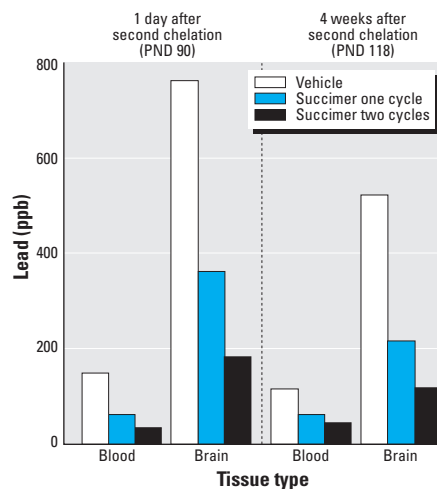


Figure 5. Additional benefit of the second regimen of succimer treatment (vs. one regimen and vehicle treatments) in reducing blood and brain lead levels, as a function of time since the second regimen ended (see text for details).

chelation for the medium ($p = 0.05$) and high ($p = 0.0003$) exposure groups. For the low lead exposure group, the lead levels were very low at both time points and there was no difference in the average lead levels at the two time points. Thus, a significant rebound in blood lead levels, but not brain lead, occurred after the second chelation, similar to the pattern after the first succimer regimen.

A significant two-way interaction between treatment and lead exposure group was found [$F(4, 144) = 5.77, p = 0.0002$], as well as a borderline three-way interaction among treatment, lead exposure, and time [$F(4, 137) = 2.93, p = 0.056$]. When assessed immediately after the second regimen (PND90), all three exposure groups derived a significant benefit of the second regimen (vs. one regimen; all $p < 0.007$), although there were subtle differences in the reduction in tissue lead relative to vehicle as a function of lead exposure. When assessed 4 weeks later (PND118), the benefit of a second chelation regimen (vs. one regimen) was still apparent in the low and medium groups but not the high exposure group (Figure 6). This finding appears to reflect primarily the rebounding of blood lead over the 4 weeks after the second chelation, which was greatest for the high-lead group.

Discussion

The results of this study provide important insights into the efficacy of succimer for the treatment of lead poisoning. First, both succimer regimens were significantly more effective than vehicle treatment in lowering lead levels in both blood and brain, although the reductions in brain lead temporally lagged behind reductions in blood lead over both treatments. In addition, a rebounding of lead

levels was seen in blood but not brain after cessation of each chelation regimen. Each of these conclusions is discussed below.

Effectiveness of succimer treatment: blood versus brain. One regimen was significantly superior to vehicle treatment in lowering lead levels in both blood and brain across the entire 8-week follow-up period. Similarly, a second succimer regimen offered significant additional benefit relative to one regimen for both tissues across the 4-week follow-up period. However, several findings revealed that succimer-induced reductions in brain lead lagged behind treatment reductions in blood lead. First, when assessed immediately after the first round of chelation, the succimer-induced reduction in lead levels (relative to vehicle levels) was greater for blood than for brain. The finding that a single chelation regimen is less effective in reducing brain lead than blood lead has been observed in other studies using either Versenate or succimer (Cremin et al. 1999; Flora et al. 1995; Seaton et al. 1999; Smith et al. 1998). This effect likely reflects two factors: First, lead in the blood is more accessible for chelation than is lead in the brain, based on the indications that chelators remove lead primarily by forming a soluble lead chelate that can be more readily eliminated via urinary or fecal routes, and by creating increased concentration gradients that favor lead efflux from tissues into the circulation. A second contributing factor is the inherently different toxicokinetics of lead in blood and brain. This phenomenon is best illustrated by the relatively slow rate of lead reduction in brain compared with blood of vehicle-treated animals over the entire study period (Figure 2). It follows, therefore, that the most effective means of reducing lead levels in well-perfused tissues exhibiting slow lead toxicokinetics (brain), as well as poorly perfused tissues (e.g., the skeleton), is to maximize the lead concentration gradient between these tissues and blood for prolonged periods of time, thereby favoring continued efflux of lead into the circulation and elimination via urinary or fecal routes.

When assessed at the end of the study (PND118), the added benefit of the second succimer regimen was more apparent for brain lead levels than for blood lead levels. This pattern also reflects the lag of brain lead reduction (relative to blood) and the different lead toxicokinetics of the two tissues. Because of the slower rate of lead efflux from brain, lead levels in this tissue were still elevated after the first regimen, whereas at this time blood lead levels were very low and appeared to have reached an asymptote. This apparent “floor” effect may be the result of both a lower amount of chelatable lead in blood and also blood lead toxicokinetics (i.e., the relative rates of lead influx to blood from other tissues vs. the rate of efflux of lead

out of the circulation). Nevertheless, the finding that the second succimer regimen offered a significant benefit in terms of reducing lead levels in both blood and brain, at all exposure intensities, is important because it indicates that multiple chelation regimens continue to lower brain lead levels, even at time points when blood lead levels have appeared to “stabilize” at a relatively low level. This observation may be particularly important in the clinical management of lead-poisoned children, because it suggests that chelation treatment may need to continue past the point at which blood lead levels have reached an acceptably low level, to achieve the maximal benefit of the treatment on brain lead levels, the primary goal in lessening cognitive dysfunction.

Experimental studies in rodents and non-human primates have yielded different results concerning the efficacy of succimer in reducing lead in blood and brain. For example, Cory-Slechta (1988), Flora et al. (1995), and Smith et al. (1998) found that succimer significantly reduced both blood and brain lead levels in rats. In contrast, a recent study in adult rhesus monkeys found that succimer treatment measurably reduced lead levels in blood but not brain (Cremin et al. 1999). The recent studies of succimer efficacy that included cognitive assessments have also yielded inconsistencies between species. Cognitive functioning was not improved by succimer treatment in the recent TLC clinical trials with children (Rogan et al. 2001), whereas a significant benefit was seen in recent studies with juvenile nonhuman primate (Laughlin and Smith 2001; Laughlin et al. 1999) and rat (Stangle et al. 2003) models of childhood lead exposure. The basis for these inconsistencies between the rodent and primate studies is not clear. However, two possibly important factors may be differences in the lead exposure history of the subjects and/or the functional duration of succimer treatment (i.e., considering species differences in metabolic rate). In particular, the results presented here suggest that chelation treatment may not have been of sufficient duration in the human and adult nonhuman primate studies to be maximally effective in terms of reducing brain lead levels.

Rebounding of lead levels after cessation of chelation. We observed a significant increase (i.e., rebound) in blood lead levels but not brain lead levels after both chelation regimens. Rebounds in blood lead levels have been reported after succimer treatment in non-human primates (Smith et al. 2000b) and in humans (Chisolm 2000; Graziano et al. 1985, 1988, 1992; Liebelt et al. 1994; Liu et al. 2002; Rogan et al. 2001). The consistency of this rebound across laboratory (rodent and primate) and clinical studies, including the recent TLC study (Rogan et al. 2001), demonstrate the importance of mobilized lead

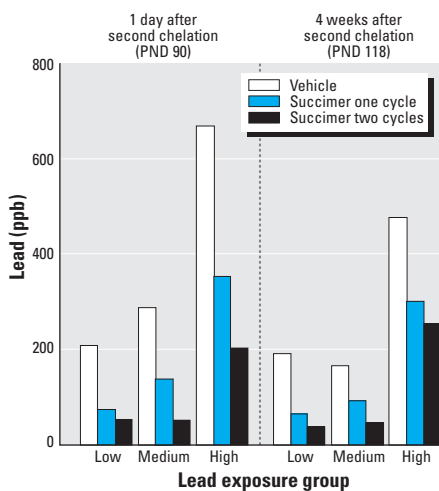


Figure 6. Additional benefit of the second regimen of succimer treatment (vs. one regimen and vehicle treatments) in reducing tissue lead (blood and brain combined), as a function of both the intensity of prior lead exposure and time since the second regimen ended (see text for details).

from endogenous sources such as the skeleton as a predominant contributor to the rebound, although reexposure to environmental lead may also have occurred in the clinical studies.

The rebound in blood lead levels observed here was not accompanied by a rebound in brain lead levels after either one or two cycles of succimer. The most likely explanation for this is that the lead concentration gradient between the blood and the brain postchelation continued to favor brain lead efflux, because of the slower reduction in brain lead levels relative to blood lead levels. Nonetheless, it is likely that the rebound in blood lead levels may have reduced the rate of decline in brain lead levels by reducing the concentration gradient between blood and brain lead postchelation. However, the larger within-group variance in brain lead levels compared with blood lead levels may have limited our ability to detect a rebound in brain lead levels.

Clinical implications. In clinical practice, decisions about chelation therapy are based on blood lead levels. However, the present results raise concerns about the adequacy of this practice. As noted above, the present findings demonstrate that after cessation of lead exposure, reductions in brain lead levels lag behind blood lead reductions in both nonchelated and chelated animals, though particularly in the latter. This finding is consistent with results from earlier rodent and nonhuman primate studies (Cremin et al. 1999; Smith et al. 1998). An obvious consequence of this lag is that brain lead levels are likely to still be elevated at times that blood leads have reached some lower value that may no longer indicate the need for additional chelation. For example, consider the data for the low and medium exposure groups in the present study (the range of blood lead levels that approximates those seen clinically): Immediately after the first chelation treatment (PND62), all 15 animals in these two exposure groups had blood lead levels at or below the analytic detection limit (5 µg/dL), whereas their brain lead levels remained quite elevated and to varying degrees (i.e., mean, 423 ± 197; range, 200–941 ng/g, compared with expected brain lead levels in non-lead-exposed animals of ~50 ng/g dry weight). These findings, as a group, point to serious limitations in using blood lead level as an indicator of brain lead when making decisions about the need for chelation therapy, particularly after a single chelation regimen when decisions are being made about the need for additional regimens.

Our findings have implications for the clinical use of succimer and its potential to ameliorate lead-induced cognitive dysfunction. Some studies have suggested that declines in blood lead levels in children are associated with improved cognitive outcomes (Ruff et al. 1993; Tong et al. 1998). Given the present

finding that succimer treatment reduced blood and brain lead levels significantly faster than did vehicle treatment (i.e., simple abatement), it follows that therapeutic use of the drug would reduce the amount of time that brain lead levels are elevated. As a result, the severity of the cognitive dysfunction should be reduced, based on evidence that lead interferes with brain development (e.g., Wilson et al. 2000). However, the TLC study, which is the only clinical study to date examining neuropsychologic functioning in lead-exposed subjects receiving succimer (Rogan et al. 2001), was unable to detect a significant benefit of succimer in terms of cognitive outcomes (relative to placebo), despite a significant lowering of blood lead levels in the treated subjects. The present findings suggest that although the treatment regimen used in the TLC study was sufficient to reduce blood lead levels below the level of concern in that study (15 µg/dL), it is likely that brain lead levels remained elevated after the cessation of treatment, because of the substantial lag in reduction of brain lead compared with blood lead levels. In the present study, the average blood lead level before receiving a second course of succimer treatment was < 15 µg/dL for all exposure groups; yet this second regimen produced a very significant further reduction in lead levels in both blood and brain for all groups. Thus, the present results suggest that treatment regimens may need to extend beyond the point at which blood lead levels have dropped to some “acceptable” low level in order to achieve the greatest possible benefit in terms of brain lead reduction and hence in terms of minimizing cognitive dysfunction.

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