Effects of calcium disodium EDTA and meso-2,3-dimercaptosuccinic acid on tissue concentrations of lead for use in treatment of calves with experimentally induced lead toxicosis

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Objective—To compare the efficacy of calcium disodium EDTA ($CaNa_2EDTA$) and meso-2,3-dimercaptosuccinic acid (DMSA) in reducing concentrations of lead in selected tissues for use in treatment of calves with experimentally induced lead toxicosis.

Animals—19 sexually intact male Holstein calves that weighed 35 to 60 kg.

Procedure—Calves were randomly assigned to 1 of 5 treatment groups: group 1, control calves; group 2, lead only; group 3, lead and EDTA; group 4, lead and DMSA; and group 5, lead, EDTA, and DMSA. Calves in groups 2 to 5 were dosed daily with lead (5 mg/kg, PO) for 10 days. Doses of EDTA (100 mg/kg) and DMSA (25 mg/kg) were administered IV once daily for 4 consecutive days beginning on day 11. Effects of the chelators on lead concentrations in the liver, kidneys, testes, muscles, bones, and brain were compared among the various groups.

Results—Compared with the effects of EDTA, DMSA greatly reduced lead concentrations in renal and hepatic tissues. We did not detect significant differences for the effects of EDTA or DMSA on lead concentrations in the testes; there was an adverse interaction of EDTA with DMSA that caused an increase in lead concentrations in the testes.

Conclusions and Clinical Relevance—DMSA is much more effective than EDTA in removing lead from renal and hepatic tissues in calves. Use of DMSA in calves with lead intoxication appears to be a viable treatment option. Combining DMSA and EDTA as a treatment modality in calves did not offer any advantages. (*Am J Vet Res* 2003;64:672–676)

Lead toxicosis is still one of the most commonly reported heavy metal toxicoses in domestic animals.[1-4] Major clinical signs of lead toxicosis in cattle relate to the gastrointestinal tract or CNS and can include initial constipation followed by diarrhea (which may contain blood), abdominal pain, weak or abolished rumen motility, blindness, ataxia, dullness, lethargy, muscle fasciculations, and convulsions.[4,5] In other studies,[5-7] investigators have documented that extremely low doses of lead are capable of causing lead toxicosis in young milk-fed calves and susceptibility of such calves can be considerably affected by diets containing hay or grain. Dietary components in hay and grain can greatly reduce the bioavailability of lead by binding to it, leading to much lower tissue concentrations of lead than those seen in calves fed solely a diet of milk replacer.[6] Diagnostically, animals are considered to have lead toxicosis when compatible clinical signs are coupled with renal or hepatic concentrations of lead ≥ 10 µg/g or blood concentrations of lead ≥ 0.35 mg/L.[4]

Nephrotoxic effects and concerns about tissue residues of EDTA have diminished the use of EDTA as a treatment for food-producing animals with lead toxicosis. Currently, none of the chelating agents are approved by the FDA for treatment of cattle in the United States with lead toxicosis. Because of its relatively low toxicity and improved margin of safety,[8,9] **meso-2,3-dimercaptosuccinic acid (DMSA)** has become the treatment of choice for humans with lead toxicosis.[10,11] Several studies[10,12-20] have examined the efficacy of EDTA and DMSA with respect to their ability to remove lead from various tissues in a number of species. Currently, DMSA is being used in dogs, cats, and birds for the treatment of toxicosis attributable to lead and other heavy metals.[2,8,12] However, little is known about the use of DMSA in food-producing animals. To our knowledge, investigators have not compared the use of EDTA and DMSA in calves. Therefore, the objective of the study reported here was to compare the efficacy of **calcium disodium EDTA ($CaNa_2EDTA$)** and DMSA, alone and in combination, as treatment to reduce concentrations of lead in selected tissues in calves with experimentally induced lead toxicosis.

Materials and Methods

Animals—Nineteen sexually intact male Holstein calves that weighed 35 to 60 kg were purchased from a local auction market for use in the study. Calves were housed in groups of 3 or 4 in indoor pens (4.9 × 6.1 m). Each pen had a concrete floor and galvanized metal poles for the sides. Each pen was bedded to a depth of 10 to 12 cm with hardwood shavings. The nutritional needs of each calf were met by feeding them a commercial milk replacer[a] twice daily via nurse bottles; amount of milk replacer fed to each calf was determined on

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the basis of body weight. The milk replacer was balanced for all known dietary requirements as determined by the National Research Council.[21] Additionally, fresh water and small amounts of high-quality alfalfa hay were available ad libitum throughout the study. Temperature was maintained at 22 ± 2°C, and lighting was controlled to provide 14 hours of light and 10 hours of darkness per day.

At the beginning of this study, health status of each calf was evaluated by means of physical examination and a CBC. Calves were housed and fed under the aforementioned conditions for 7 days to ensure proper acclimation, growth, and health. All calves were judged to be in good health prior to the administration of lead. All procedures involving the use of animals in this study were approved by the Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University.

Experimental protocol—The 19 calves were randomly assigned to 1 of 5 treatment groups: group 1, control calves (no lead or chelators); group 2, lead only; group 3, lead and EDTA; group 4, lead and DMSA; and group 5, lead and EDTA and DMSA. There were 3 calves in group 1, whereas all other groups had 4 calves/group.

Dextrose[b] and sodium bicarbonate[c] were purchased and used to make solutions (2.5% dextrose and 5% sodium bicarbonate) that were formulated in our laboratory; we used sterile deionized water as the vehicle. Reagent grade lead acetate[d] was dissolved in 2.5% dextrose solution and administered orally via a nurse bottle daily for 10 days to calves in groups 2 to 5. Each calf in those groups received lead at a rate of 1 mL/kg (ie, daily dose of 5 mg of lead/kg). This dosage regimen was selected on the basis that it induces lead toxicosis in calves within 7 to 10 days.[5,6] The lead solution was administered at a time point midway between the 2 daily feedings of milk replacer to maximize lead absorption. Calves in group 1 received an equivalent amount of dextrose solution at the same time as the lead administration in calves in the other groups.

Chelation treatment was administered for 4 consecutive days beginning on day 11. Solutions of both chelators were prepared fresh daily by dissolving them in a sterile solution of 5% sodium bicarbonate to maintain pH at approximately 7.0 to 7.2. To maintain consistency with dosage ranges reported in the literature,[2-4,13,18] $CaNa_2EDTA$[e] (ie, EDTA) and DMSA[f] were administered IV once daily at dosages of 100 and 25 mg/kg (1.0 and 0.5 mL/kg), respectively. The EDTA and DMSA were administered alone (groups 3 and 4) or in combination (group 5).

On the day after cessation of chelation treatment (day 15), all calves were euthanized by IV administration of a lethal dose of sodium pentobarbital (60 mg/kg). Calves were then immediately exsanguinated. Tissue samples were collected from the liver (caudal portion of the left lobe), left kidney, left testis, biceps femoris muscle, brain (parietal region of the left cerebral cortex), and rib (5-cm portion from the middle of the left fourth rib). All muscle and periosteum were immediately stripped from the rib to provide a clean sample of bone. Tissue samples were kept frozen at –20°C until analyzed for lead content.

The primary objective of the study was to determine the comparative ability of EDTA and DMSA to reduce lead concentrations in various tissues. The ability of EDTA and DMSA to decrease lead concentrations in the blood has been documented[10,11,13,18]; thus, lead concentrations in the blood were not evaluated in the study reported here.

Analysis of lead concentrations—Soft-tissue samples were thawed and finely minced with scissors. Aliquots (approx 1 g) were weighed and then digested in a 5:1 mixture of concentrated nitric acid (70% perchloric acid) by use of a slight modification of the wet-ashing technique of Ihnat and Miller[22]. The section of rib was cut into small chips, and aliquots (1 to 2 g) were digested in the same manner as the soft-tissue samples. Briefly, we used 10 mL of acid mixture/g of tissue. Samples were heated at 150°C for 2 hours, with nitric acid added as needed in a drop-wise manner to prevent charring until the organic matter was completely destroyed. Lead concentrations were determined by atomic absorption spectrophotometry[g] by use of a graphite furnace. Limit of detection for this analysis was 5 ng/g, and recovery for spiked samples was > 90%.

Statistical analysis—A statistical program[h] was used to perform a 2-way ANOVA to test for main effects of EDTA and DMSA, along with their interactions, on lead concentrations measured in each tissue. Plots of standardized residuals were used to assess adequacy of the model. Once adequacy of the model was established, all response variables were logarith-

Table 1—Effect of chelation treatment on lead concentrations in various tissues of calves orally administered lead acetate*

Group	Liver	Kidney	Testis	Muscle	Bone	Brain
Control† (No lead)	0.3 (0.2–0.5)	0.7 (0.3–1.8)	0.02 (0.01–0.03)	0.01 (0.01–0.03)	10.9 (7.7–15.5)	0.01 (0.01–0.03)
Lead only	4.2 (3.0–5.8)	15.9 (7.1–35.8)	0.20 (0.13–0.33)	0.04 (0.02–0.10)	39.9 (29.4–54.1)	0.33 (0.18–0.59)
Lead and EDTA (100 mg/kg)	4.1 (2.9–5.7)	26.4 (11.8–59.3)	0.19 (0.11–0.31)	0.06 (0.03–0.14)	54.3 (40.1–73.7)	0.19 (0.10–0.34)
Lead and DMSA (25 mg/kg)	2.5‡ (1.8–3.5)	2.1§ (0.9–4.6)	0.11 (0.07–0.18)	0.07 (0.03–0.17)	49.0 (36.1–66.4)	0.32 (0.18–0.58)
Lead and EDTA (100 mg/kg) and DMSA (25 mg/kg)	2.4 (1.7–3.3)	7.3 (3.3–16.4)	0.29¶ (0.18–0.48)	0.08 (0.03–0.20)	50.7 (37.4–68.8)	0.17 (0.09–0.30)

Values reported are geometric mean (95% confidence interval) parts per million (ie, µg/g) for lead concentrations.

*Calves were administered lead (5 mg/kg, PO, q 24 h) as lead acetate for 10 days, and treatment was administered on days 11 through 14. †Mean concentrations for tissues obtained from calves in the control group differed significantly ($P < 0.001$) from concentrations in tissues obtained from all lead-treated calves. ‡Value differs significantly ($P = 0.012$) from value for lead-only group. §Value differs significantly ($P = 0.002$) from value for lead-only group. ¶Value differs significantly ($P = 0.021$) from value for lead and DMSA group.

DMSA = Meso-2,3-dimercaptosuccinic acid.

mically transformed to stabilize variances. Means were back-transformed after analysis. Therefore, geometric means and 95% confidence intervals were reported. Significant interactions were further investigated to test for simple main effects (effect of 1 factor within a single level of the other factor). Interaction terms evaluated whether the effect of EDTA on lead concentration in a specific tissue was the same with or without concurrent administration of DMSA. Significance was defined as values of $P \leq 0.05$.

Results

All calves in the study that received lead developed mild diarrhea and dark feces after 3 or 4 feedings of lead acetate. Three calves (2 in group 3, 1 in group 4) had evidence of frank blood (grossly visible) in their feces. Occult blood tests were not performed. One calf in group 2 developed signs of blindness (evident as a lack of a menace reflex and bumping into objects and other calves), lethargy, and hypoglossal paresis (did not suckle well during the last 2 or 3 feedings of lead). Statistical analysis of each of the tissue samples revealed a significant difference ($P < 0.001$) in lead concentration in calves that received lead (groups 2 to 5), compared with control calves of group 1 (**Table 1**).

Administration of DMSA significantly ($P = 0.002$) decreased lead concentrations in renal tissues (15.9 to 2.1 ppm), whereas EDTA treatments increased, but not significantly ($P = 0.056$), lead concentrations in renal tissues (15.9 to 26.4 ppm; Table 1). With respect to the renal tissues, we did not detect significant interactions between EDTA and DMSA.

Administration of DMSA significantly ($P = 0.012$) decreased lead concentrations in hepatic tissues (4.2 to 2.5 ppm). Lead concentrations in hepatic tissues of calves treated with EDTA were essentially unchanged from those in the lead-only group (ie, group 2), and we did not detect a significant interaction between EDTA and DMSA (Table 1).

We did not detect significant main effects of EDTA or DMSA on lead concentrations in testicular tissues. However, analysis of the data revealed an interaction between EDTA and DMSA, which was not significant ($P = 0.065$; Table 1). Further statistical analysis (ie, examining the effect of 1 factor within a single level of the other factor) revealed that when EDTA was combined with DMSA, lead concentration in the testis increased significantly ($P = 0.021$) from 0.11 to 0.29 ppm, but when EDTA was administered alone (ie, without concurrent administration of DMSA), there was not a significant ($P = 0.826$) effect (data not shown).

We did not detect significant main effects of EDTA or DMSA or any significant interactions between EDTA and DMSA on lead concentrations in muscle, bone, or brain tissues (Table 1).

Discussion

Analysis of results of the study reported here confirm those of other studies[3,5] in that they document that young, milk-fed calves are highly susceptible to lead toxicosis, as evidenced by the fact that within 10 days after initiation of oral administration of lead doses, all lead-treated calves developed several clinical signs compatible with lead intoxication. Furthermore, statistical analysis revealed that tissue lead concentrations of lead-exposed calves were significantly higher than those of control calves of group 1. Additionally, analysis of lead concentrations revealed a mean concentration in renal tissues of 15.9 μg/g in group 2 (administered lead but no treatment), which is much greater than the value of 10 μg/g considered to be a diagnostic standard for lead toxicosis.[4,23] Taken in combination, these facts indicate that the dosing regimen we used resulted in the desired outcome of clinical signs of lead toxicosis in the lead-exposed calves.

Diet has a major effect on tissue concentrations of lead and the development of lead toxicosis; thus, it should be mentioned that calves in this study were fed milk replacer and small amounts of alfalfa hay but did not have access to grain or other concentrate. This dietary modification may explain the minor differences seen in tissue lead concentrations in calves from this study and those reported in another study.[5]

Under the conditions for the study reported here, DMSA was much more effective than EDTA in removing lead from the kidneys and liver. In sharp contrast, administration of EDTA significantly increased lead concentrations in the kidneys to nearly double those for the lead control group, whereas lead concentrations in the liver remained unchanged from those for the lead control group. Because the kidneys and liver are edible tissues, use of DMSA to treat calves with lead toxicosis would provide an added benefit for food safety. Results of our study for the use of DMSA in calves are similar to those in studies[14,15,18,24] involving the use of DMSA in rats and mice, which resulted in large decreases in lead burdens in the kidneys and liver. However, with respect to EDTA administration, results obtained in calves of our study differed markedly from those obtained in rodents. In studies involving the use of rodents, EDTA significantly reduced lead concentrations in the kidneys and liver,[15,18] although other investigators[13] detected at least a temporary increase in lead concentrations in the liver. In the latter study, hepatic lead concentrations increased from days 1 to 3 of EDTA treatment and then decreased below control values on day 4 or 5. The variability in results among chelators or among species makes it difficult to predict the effects of chelators on lead concentrations in specific organs. This differing effect on renal lead concentration in calves may be associated with redistribution of lead from other tissues, or it may be that a longer or repeated course of treatment is needed to decrease lead concentrations in renal tissues of cattle. Frequency of chelator administration may also account for the difference. Additional studies will be required to answer this question.

Because the deposition of lead in brain tissue causes severe clinical disease, decreasing the concentration of lead in that organ has been a primary goal of chelation therapy in humans[10,15,25,26] and other animals.[14,16,25-27] In studies involving the use of rats[10,14,24,25] and mice,[15] DMSA decreased lead concentrations in the brain, but the effect of EDTA chelation on lead concentrations in the brain yielded mixed results.[10,13,15] Data from the study reported here was not conclusive with regard to the superiority of 1 chelator over the other for reduc-

ing lead concentrations in the brain, as determined by the fact that there was not a significant difference between the effects of EDTA and DMSA.

Chelation therapy is based on the premise that it will decrease the total body burden of lead by causing substantial excretion of lead via the urine. Indeed, the efficacy of EDTA and DMSA in causing substantial increases in urinary excretion of lead has been documented for a range of species by several investigators.[2,3,11,14,18,20] Flora et al[18] reported significant decreases in lead concentrations in the liver and kidneys, coupled with a significant increase in urinary excretion of lead in rats treated with DMSA or EDTA for 5 days. Smith et al[20] reported similar findings for hepatic tissues of monkeys treated for 5 days with DMSA. Because data on urinary excretion were not obtained in the study reported here, some caution must be used in interpreting tissue concentrations of lead. However, on the basis of results of the other aforementioned studies, it seems reasonable to conclude that the decreased renal and hepatic concentrations of lead detected in our DMSA-treated calves were largely attributable to enhanced urinary excretion, although it is also possible that some of the decrease could have been attributable to tissue redistribution.

Consideration has been given to combining 2 chelators (EDTA and DMSA) for simultaneous administration to animals in hopes of obtaining an additive effect for depleting tissues of lead.[16-18] In the study reported here, administration of a combination of DMSA and EDTA as a treatment modality did not decrease lead concentrations in any organ below those achieved by administration of either chelator alone. In fact, there appeared to be an adverse interaction of the 2 chelators with respect to lead concentration in testicular tissues, because lead concentrations increased in that tissue when the 2 chelators were used together. Other researchers[16,18] obtained an improved (additive) effect of combined chelation therapy in rats for the liver and kidneys, whereas in another study,[17] investigators detected a beneficial effect on lead concentrations in the liver but an adverse effect on lead concentrations in the kidneys. In another dosing study[19] that involved the use of rats, it was found that use of a combination of EDTA and DMSA for two 5-day series of treatments yielded the more effective recovery from lead-induced testicular disorders.

Increased lead concentrations in testicular tissue that were detected in this study raise several questions. Although the causes are not clear, redistribution from other tissues during the course of chelation therapy is 1 possible explanation. This phenomenon has been reported[2,10,13,18] for other tissues. Additional possibilities include vascular damage or injury to other cellular components of the testes as a result of lead or chelating agents, which could have led to increased accumulation or decreased excretion of lead from testicular tissue. Because tissues were not obtained for histologic examination, these latter possibilities cannot be evaluated. Furthermore, increased lead concentrations in the testes may have implications for fertility or other reproductive problems in affected animals. In a study[28] in which investigators administered lead acetate to rabbits, they did not detect evidence of adverse effects on spermatozoa counts, spermatozoa morphology, or histologic characteristics of the testis. Whether the same holds true in cattle has not been determined. Additional studies must be conducted to determine whether more prolonged combination chelation therapy in calves can achieve the effect observed in another study.[19] Although additional adverse interactions in other tissues were not observed in our study, this finding warrants further investigation to determine whether there may be other adverse interactions between EDTA and DMSA.

Lead concentration in muscles is generally quite low,[5] and data from the study reported here support this finding. Given this low tissue burden, it is difficult to document that chelating agents have much effect on lead concentrations in muscle tissue. From a public health perspective, there is seldom any danger to humans consuming meat (ie, muscle tissue) from calves with lead toxicosis,[1,29] because the total dose of lead from this source would be quite small.

Bone is recognized as a tissue used to store a considerable portion of the body burden of lead.[5] Furthermore, several studies[13,16,27] have revealed that at least some portion of lead in bones is accessible to chelators, as evidenced by the fact that EDTA and DMSA can reduce lead concentrations in bone. Therefore, the results obtained in the study reported here were surprising, because we did not detect a decrease in lead concentrations in bone, nor was there even a pattern toward a decrease in lead concentration. Whether this disparate result was associated with species differences in lead kinetics, the chelator dosage regimen (single daily dose vs multiple doses per day), length of chelation treatment, or lead dynamics in various bones (femur in rats vs rib in cattle) cannot be determined from the data available. However, differing kinetics in flat bones versus long bones is a less likely explanation, because studies[18,30,31] in which investigators examined lead removal from the skull (flat bone) and femurs (long bone) documented that chelators are capable of removing lead from both types of bone. It is also possible that the slightly higher lead concentrations seen in bone are a result of redistribution from soft tissues to bone during chelation and redeposition.

The range of species in which DMSA is a clinically useful chelating agent continues to grow.[2,8,12,20] The study reported here adds 1 more species to that list, because DMSA appears to be a viable treatment option in calves with lead toxicosis. However, additional studies in calves are needed to determine the ability of DMSA to eliminate lead from the body via the urine, ameliorate clinical signs of lead toxicosis, and assess the toxic effects of DMSA in this species. Cost will also have a major bearing on its use in food-producing animals.

[a]Land O'Lakes milk replacer, Southern States Cooperative, Richmond, Va.
[b]Dextrose, Sigma Chemical Co, St Louis, Mo.
[c]Sodium bicarbonate, Sigma Chemical Co, St Louis, Mo.
[d]Lead acetate, Sigma Chemical Co, St Louis, Mo.
[e]Calcium disodium EDTA, Sigma Chemical Co, St Louis, Mo.
[f]Meso-2,3-dimercaptosuccinic acid, Sigma Chemical Co, St Louis, Mo.

[g]Varian instruments procedures manual, Varian Optical Spectroscopy Instruments, Wood Dale, Ill.
[h]SAS, version 8.1, SAS Institute Inc, Cary, NC.

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