Serum elimination profiles of methyllycaconitine and deltaline in cattle following oral administration of larkspur (*Delphinium barbeyi*)

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**Objective**—To describe the simple elimination kinetics of methyllycaconitine (MLA) and deltaline and evaluate the heart rate response in cattle following oral administration of larkspur.

**Animals**—5 healthy Angus steers that were habituated to metabolism crates.

**Procedures**—Tall larkspur (*Delphinium barbeyi*) in the early flowering stage was collected, dried, and ground. Each steer received a single dose of larkspur that was equivalent to 10.4 mg of MLA/kg and 11.0 mg of deltaline/kg via oral administration. Steers were housed in metabolism crates during a 96-hour period following larkspur administration; heart rate was monitored continuously, and blood samples were collected periodically for analysis of serum MLA and deltaline concentrations as well as assessment of pharmacokinetic parameters.

**Results**—No overt clinical signs of poisoning developed in any steer during the experiment. Mean ± SE heart rate reached a maximum of 79.0 ± 5.0 beats/min at 17 hours after larkspur administration. Serum MLA concentration was correlated directly with heart rate. Mean times to maximal serum concentration of MLA and deltaline were 8.8 ± 1.2 hours and 5.0 ± 0.6 hours, respectively. Mean elimination half-life values for MLA and deltaline were 20.5 ± 4.1 hours and 8.2 ± 0.6 hours, respectively.

**Conclusions and Clinical Relevance**—Following larkspur administration in 5 healthy steers, maximum serum concentrations of MLA and deltaline were detected within 10 hours, and changes in serum MLA concentration and heart rate were correlated. Results indicated that cattle that have consumed larkspur will eliminate 99% of MLA and deltaline from serum within 144 hours. (*Am J Vet Res* 2009;70:926–931)

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The toxic effects of larkspur (*Delphinium* spp) in cattle have been attributed to the norditerpenoid alkaloids that are produced by the plant and that are present in high concentrations in plant tissues. Clinical signs associated with the consumption of larkspur plants that have high concentrations of toxic alkaloids include muscle weakness and trembling, tachycardia, failure of voluntary muscular coordination, sternal recumbency followed by lateral recumbency, bloating, respiratory depression, and death. The progression of these signs is often rapid, and the clinical signs may not be observed—poisoned animals are commonly found dead. In addition to these well-documented clinical signs of larkspur poisoning, less obvious dose-dependent changes in heart rate and in the electrically evoked electromyographic response of the external anal sphincter have been recently identified. These changes are detectable within 24 hours after administration of low doses of larkspur when other signs are minimal or have not yet developed.

Larkspur contains MSAL-type and MDL-type norditerpenoid alkaloids, of which the MSAL-type alkaloids are the more toxic. Members of the MSAL-type alkaloids include MLA, nudicauline, and 14-deacetylnudicauline. Plants that have high concentrations of MSAL-type alkaloids are thought to have the most toxic effects in cattle, and the concentrations of these alkaloids have been used for the prediction of plant toxicity. Members of the less toxic MDL-type alkaloids include deltaline and 14-O-acetyldictyocarpine.
Of the norditerpenoid alkaloids, the elimination kinetics and toxic effects of MLA have been most extensively studied. Turek et al\textsuperscript{10} reported that the serum $t_{1/2}$ of MLA in rats following oral administration of the alkaloid was 408 minutes, whereas the $t_{1/2}$ of MLA following IV administration of the alkaloid was only 18 minutes. Purified MLA has an LD$_{50}$ of 4.5 mg/kg in mice.\textsuperscript{11} By use of a mouse bioassay, the simple elimination kinetics of MLA (administered IV) in serum and kidney, liver, brain, and muscle tissues were evaluated, and the $t_{1/2}$s for those samples were 17.5, 21.1, 22.0, 15.6, and 10.2 minutes, respectively.\textsuperscript{12} In sheep, MLA is rapidly eliminated with a $t_{1/2}$ of 20 minutes after IV injection.\textsuperscript{13} More recently, Welch et al\textsuperscript{13} determined the kinetics of purified MLA in brain and muscle tissues after IV administration of the alkaloid alone and in combination with other larkspur alkaloids in mice. In mice, IV administration of MLA in combination with deltaline, 14-O-acetyldictyocarpine, or lycocotine did not alter the bioavailability or clearance of the alkaloids.\textsuperscript{13} In comparison, toxicokinetics and possible synergisms of the MDL-type norditerpenoid alkaloids have been investigated to a much lesser extent. Following IV administration in mice, the LD$_{50}$ of deltaline (an MDL-type alkaloid that lacks the $N^2$-methylsuccinyl)anthranilic acid ester at the C-18 position) was 115 mg/kg.\textsuperscript{13} The serum $t_{1/2}$ of deltaline is approximately 20 minutes after IV injection in sheep.\textsuperscript{9,11} There is little information available on the simple elimination kinetics of MLA and deltaline or on the physiologic effects of these alkaloids following oral administration to cattle, even though grazing cattle are the single species known to be fatally intoxicated by larkspurs. The purpose of the study reported here was to describe the simple elimination kinetics of MLA and deltaline and evaluate the heart rate response in cattle during a 96-hour period following oral administration of dried ground tall larkspur.

**Materials and Methods**

Plant material—Tall larkspur (*Delphinium barbeyi*) in the early flowering stage was collected during July 2003 near Manti, Utah (latitude, 39° 03.154’ N; longitude, 111° 30.752’ W; Poisonous Plant Research Laboratory collection No. 03-12), at an elevation of approximately 3,000 m above sea level. A voucher specimen was deposited at the Utah State University Herbarium (No. 237494). The plant material was air-dried, ground until sufficiently fine to pass through a 2.38-mm mesh, and mixed by use of a grinder-mixer.\textsuperscript{9} After processing, the plant material was stored in plastic bags away from direct light at room temperature (approx 9°C) until use.

Alkaloid analysis—Dried ground larkspur samples (5 replicates) were extracted\textsuperscript{7} and analyzed for alkaloid content by use of methods that have been previously described.\textsuperscript{14} Reserpine\textsuperscript{8} at a concentration of 500 µg was used as an internal standard. Individual alkaloid concentrations were quantified from peak areas in ion chromatograms generated from their respective protonated ions in reference to calibration curves. These alkaloid concentrations were used for the calculation of the doses of alkaloids in the form of dried ground larkspur.

Serum samples were stored and extracted as previously described.\textsuperscript{11} Mass spectrometry analyses of the serum samples and standards were also performed as previously described.\textsuperscript{13} The extract of this larkspur collection from the year 2003 contained 16 mg of norditerpenoid alkaloids/g, of which 7 mg/g represented the MDL-type alkaloids and 9 mg/g represented the MSAL-type alkaloids.\textsuperscript{3}

Cattle—Five black Angus steers (mean ± SE weight, 596 ± 10 kg) were used in the study. All animal work was done under veterinary supervision with the approval of the Utah State University Institutional Animal Care and Use Committee. The steers were maintained on alfalfa grass hay with a mineral supplement for at least 3 weeks before and during the experiments. The steers were trained to the use of a halter, gentled, and handled frequently so that they were highly tractable; these animals were habituated to the metabolism crates.

**Study design**—In the first experiment, each steer received a single dose of dried ground larkspur orally; the dose provided 10.4 mg of MLA/kg and 11.0 mg of deltaline/kg. During a 96-hour period following larkspur administration, heart rate was monitored continuously and blood samples were collected periodically for analysis of serum MLA and deltaline concentrations. Three weeks after administration of dried ground larkspur, the experiment was repeated with the exception that the larkspur was replaced with similar amounts of dried ground grass hay. In this second experiment, the steers were similarly monitored during a 72-hour period, thereby allowing each to act as its own negative control animal.

**Procedures for the first experiment**—Eighteen hours prior to the start of each experiment, a 16-gauge indwelling catheter\textsuperscript{7} was placed in a jugular vein of each steer. To place the catheter, each steer was sedated with acepromazine maleate\textsuperscript{6} (0.05 mg/kg, IV). In the area over the jugular vein that was selected for catheter insertion, the hair was clipped and the site was washed with a surgical scrub solution\textsuperscript{9} and rinsed with 70% ethanol. After site preparation, each steer was administered 5 mL of lidocaine hydrochloride\textsuperscript{5} SC to provide local anesthesia around the site of catheter placement. The catheter was then inserted in the jugular vein. An extension tube was glued to the catheter and skin at the junction site with cyanoacrylate adhesive and sutured in place to the skin at the junction site. The extension tubing was positioned along the neck of the animal, and the port was placed between its ears; the extension tube was protected along its length with several layers of elastic-adhesive tape.\textsuperscript{7} The steer was then led into a metabolism crate, and food was withheld during the night prior to the start of the experiment.

During the day of the experiment, each steer was handled in a gentle manner; baseline weight and physiologic measurements (designated as 0-hour data) were recorded. A single dose of finely ground dried larkspur mixed in approximately 11 L of tap water was administered via oral gavage. At 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 hours, blood samples were collected at each of these times. The steers were then returned to the metabolism crate, and food was withheld during the next 24 hours.
Heart rate data are obtained for an approximately 5-minute period just prior to each of the 19 venous blood sample collections. The mean heart rate values obtained prior to the venous blood sample collections were used to assess the correlation between serum alkaloid concentrations and heart rate via Spearman correlation analysis.

The kinetic profiles of MLA and deltaline were analyzed by use of standard pharmacokinetic software. A curve-stripping procedure was used to determine the basic pharmacokinetic parameters for the rates of elimination phases of the MLA and deltaline concentration curves. The following parameters were determined for each alkaloid: $t_{1/2}^{\alpha}$ (ie, $0.693/k_{\text{elimination}}$), $C_{\text{max}}$, $T_{\text{max}}$, and AUC. A trapezoidal method was used to determine the AUC of a concentration versus time graph. Comparisons of mean values derived following control and larkspur treatments were made by use of an ANOVA followed by a Dunnett test, as indicated. Comparisons between 2 means were made by use of a 2-tailed $t$ test. For all comparisons, the limit for significance was set at a value of $P < 0.05$.

**Results**

Five steers received dried ground larkspur at a dose that was equivalent to 10.4 mg of MLA/kg and 11.0 mg of deltaline/kg via oral gavage during the first experiment and a similar amount of dried ground pasture grass hay during the second experiment. For the duration of both experiments, heart rate was recorded continuously (data not shown) and venous blood samples were collected at intervals. During the entire 96-hour period following administration of larkspur, the 5 steers developed no overt clinical signs of poisoning.

At 17 hours after administration of larkspur, mean ± SE heart rate attained a peak value of 79 ± 5 beats/min; this value was based on data from 4 steers because 1 steer had removed its monitoring leads at 17 hours, although they were subsequently reattached. The heart
rate at 17 hours in larkspur-treated cattle was significantly different from both the mean baseline heart rate in that first experiment (59 ± 2.0 beats/min; \( P = 0.004 \) \([n = 5]\)) and the mean heart rate at 17 hours among cattle receiving the control treatment (52 ± 4 beats/min; \( P = 0.005 \) \([4]\)). After reaching the maximum heart rate at 17 hours, the rate declined to a value of 54 ± 6 beats/min at 96 hours (\( n = 5 \)). A similar peak heart rate value was not detected in cattle following administration of the dried ground pasture grass hay; from 10 to 20 hours after administration of the control treatment (the period corresponding to the period of maximum heart rate in larkspur-treated cattle), mean heart rate was 56 ± 3 beats/min, and during the second experiment overall, the mean heart rate was 61 ± 2 beats/min.

Peak serum concentrations of MLA and deltaline were detected at the 10- and 6-hour time points, respectively. The peak serum MLA concentration was 305.9 ± 94.2 ng/mL, and the peak serum deltaline concentration was 476.5 ± 62.8 ng/mL (Figure 1). At 96 hours, the serum MLA concentration was 17.1 ± 8.4 ng/mL and the serum deltaline concentration was less than the limit of detection. The \( T_{max} \) values for MLA and deltaline were calculated from data obtained from all 5 steers and were 8.8 and 5.0 hours, respectively (Table 1). The \( T_{max} \) values for the 2 diterpenoid alkaloids were significantly (\( P = 0.022 \)) different. The \( t_{1/2} \) for MLA and deltaline were also significantly (\( P = 0.018 \)) different. On the basis of the data from all 5 steers, there was a significant direct correlation (Spearman \( r = 0.75 \); \( P < 0.001 \)) between serum MLA concentration and heart rate. No such correlation was detected between serum deltaline concentration and heart rate (Spearman \( r = 0.33 \); \( P = 0.2 [n = 5] \)).

**Discussion**

In a previous study \( ^{3} \) performed by our group, dose-dependent increases in heart rate and decreases in the evoked electromyographic response of the external anal sphincter were detected in cattle that received larkspur orally. On the basis of those findings, the dose of larkspur used in the present study was selected to provide an equivalent dose of alkaloids that could be safely administered to cattle without the loss of voluntary muscle coordination. The larkspur collection used in the present study contained nortriterpenoid alkaloids, of which deltaline and MLA were in greatest abundance; the dose of larkspur administered to the cattle provided the equivalent doses of 10.4 mg of MLA/kg and 11.0 mg of deltaline/kg. \( ^{3} \) This alkaloid dose was tolerated well by the 5 Angus steers, with no development of notable muscle weakness or overt clinical signs of larkspur toxicosis. In a previous pen-based study \( ^{3} \) involving the same larkspur collection, mixed-breed cattle that received this dose did develop overt voluntary muscle weakness. The lack of muscle fatigue in the present study may have been attributable to breed differences or, more likely, to the fact that confinement of the steers in metabolism crates (which limits movement) minimized exertion, thereby reducing muscle fatigue. These steers had extensive training and habituation to the experimental protocol and metabolism crates that certainly would reduce stress during each 96-hour experiment. Additional research is needed to determine the factors behind the observed differences in clinical effects between the pen-based investigation and the present study.

We have previously posed the hypothesis that MSL-type alkaloids acting at \( \alpha_{7} \)-nAChRs in the intercardiac ganglia were responsible for the increases in heart rate in cattle that are poisoned with larkspur. \( ^{3} \) In the present study, heart rate was significantly increased, compared with baseline values, at 17 hours after oral administration of larkspur. Moreover, there was a significant correlation between serum MLA concentration and heart rate. The fact that there was a correlation between serum MLA concentration, but not serum deltaline concentration, and heart rate in cattle provides support for that previous hypothesis. Methyllycaconitine is a potent and selective competitive antagonist with nanomolar affinity at \( \alpha_{7} \)-nAChRs. \( ^{10-18} \) Deltaline is a significantly less potent nicotinic receptor blocker; MLA is > 100 times as potent as deltaline at reducing compound muscle action potentials in lizard extensor digitorum longus muscle preparations. \( ^{10} \) On the basis of results of the present study and a previous dose-response study \( ^{3} \) in cattle, it is likely that the inhibition of cholinergic neurotransmission by diterpenoid alkaloids is responsible for the observed clinical effects in cattle. Further research to elucidate the actions of purified MSL-type and MDL-type alkaloids in cattle is needed.

Measurements of kinetic parameters in the present study also provided insight into the time of onset of clinical effects following larkspur ingestion and the clearance times of MLA and deltaline in cattle. Previous research at our laboratory has revealed that cattle typically collapse to recumbency at 5 to 8 hours after oral administration of dried ground larkspur. \( ^{3,20,21} \) These observations coincide with the \( T_{max} \) values calculated for MLA and deltaline. Although the kinetics of toxin elimination from cattle that are treated with fresh larkspur plant preparations, as occur when grazing cattle are poisoned, may be different, results of our study suggested that clinical intoxication correlates closely with the \( T_{max} \) values of MLA and deltaline and that clinical signs are likely to develop or be most severe at 6 or 7 hours after exposure. More work is needed to ascertain the clinical importance of these findings, as many cattle ingest larkspur over extended periods when grazing, thereby developing so-called repetitive dosing or equilibrium kinetics whereby many tissues have rela-
tively constant cyclic toxin concentrations. These animals become intoxicated when too much additional larkspur is eaten too quickly.

Although MLA is more potent than deltaline, the high serum concentrations of both alkaloids that were detected early in larkspur-treated cattle in our study may be biologically important. In experiments with mice, IV coadministration of MLA and deltaline increases the toxic effects, compared with the effects of MLA alone; the LD<sub>50</sub> of the MLA-deltaline combination is 3.1, 2.2, and 1.5 mg/kg at ratios of 1:1, 1:5, and 1:25, respectively, whereas the LD<sub>50</sub> of MLA alone is 4.4 mg/kg. These findings suggest that concentrations of both alkaloids contribute to larkspur toxicity and determine the risk of poisoning-associated clinical effects.

In the cattle of the present study, MLA was still detectable in serum at 96 hours after treatment with larkspur. Given that 7 <i>t</i><sub>1/2</sub>s are required for 99% elimination of the alkaloid from serum, it would require a period of 144 hours or 6 days to clear 99% of the MLA from the serum in these cattle. The <i>t</i><sub>1/2</sub> for MLA in our study (approx 20 hours) is considerably longer than previously reported values (approx 20 minutes).<sup>10–12</sup> One explanation for this discrepancy is species differences. Methyllycaconitine may be eliminated more slowly in cattle than it is in sheep, rats, or mice. However, caution should be taken when estimating the <i>A</i><sub>1/2</sub> and <i>t</i><sub>1/2</sub> of a compound on the basis of data derived from experiments involving oral administration because of flip-flop kinetics. A flip-flop kinetic model occurs when the absorption of a compound is much slower than its elimination, and this type of kinetic model can be generated whenever <i>A</i><sub>1/2</sub> and <i>t</i><sub>1/2</sub> are estimated as the 20-minute <i>t</i><sub>1/2</sub> for MLA in our study would be approximated. This is suggestive of flip-flop kinetics because the true elimination rate of a compound will be the same for both methods of administration. If MLA follows a flip-flop kinetic model in cattle, then the <i>t</i><sub>1/2</sub> of MLA in the present study would be approximately 3 hours—a value that is much closer to the half-life values reported previously, although not as rapid as the 20-minute <i>t</i><sub>1/2</sub>.<sup>10–12</sup> To correctly estimate <i>A</i><sub>1/2</sub> and <i>t</i><sub>1/2</sub>, data obtained from animals following oral and IV administrations are needed.<sup>22</sup> To perform an IV kinetics study in cattle, large quantities of purified MLA and deltaline would be required, which is not presently feasible. If the absorption of MLA is the rate-limiting step in cattle, then the administration of activated charcoal to decrease the absorption of MLA could be a possible treatment for larkspur poisoning; however, additional research is needed to determine whether that treatment would be effective.

In the present study, most deltaline was cleared from serum after a period equivalent to 7 <i>t</i><sub>1/2</sub>s, and although it was still present at 60 hours, the serum concentration was less than the limit of detection at 96 hours. The initial toxicity data and these relatively rapid clearances associated with deltaline (compared with clearances of the MSAL-type alkaloids) suggested that deltaline contributes little to the toxic effects of larkspur in cattle; however, results of a recent study<sup>23</sup> in mice suggest that the apparently less toxic MDL-type alkaloids enhance the toxicity of the more toxic MSAL-type alkaloids. Because of species differences, further research is needed to investigate the effects of MDL-type and MSAL-type larkspur alkaloids combined in different ratios and the role of those alkaloids in the toxic effects and toxicokinetics of larkspur in cattle. It should be noted that there are differences in the potency and binding affinities of larkspur alkaloids depending on the type of tissue and species.<sup>23,24</sup> For example, cattle are more susceptible to the toxic effects of larkspur than are sheep.<sup>9</sup> The effects of low doses of larkspur alkaloids in other species such as humans are unknown, and the possibility of an anaphylactic reaction or other response to these toxins does exist. It is suggested that in animals that have been exposed to larkspur, the time to allow complete clearance of larkspur alkaloids is 6 to 7 days.

In the 5 steers in the present study, maximum serum concentrations of MLA and deltaline were detected within a period of 10 hours after oral administration of larkspur; of the 2 alkaloids, MLA has a slower <i>t</i><sub>1/2</sub> (20.5 hours), compared with that of deltaline (8.2 hours). Because the clearance of MLA is relatively prolonged, a withdrawal time of 7 days is suggested to allow poisoned animals to eliminate larkspur-associated toxins. Given that serum MLA concentration was correlated with heart rate, development of tachycardia may be an early indicator of poisoning. Additional research is needed to better determine the biological, effects and mechanisms of MSAL and MDL interactions in intoxication and to determine why some animals or species are more susceptible to poisoning.

References: