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Serum toxicokinetics after intravenous and oral dosing of larkspur toxins in goats

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ABSTRACT

Poisoning of cattle by larkspur plants (*Delphinium* spp.) is a concern for cattle ranchers in western North America. Previous research studies have evaluated the toxicokinetic profile of multiple larkspur toxins in several livestock species. However, those studies were all performed by orally dosing plant material. Consequently some toxicokinetic parameters could not be definitively determined. In this study, we compared the serum toxicokinetic profile of the larkspur alkaloids methyllycaconitine (MLA) and deltaline in goats dosed both IV and via oral gavage. The results from this study indicate that the toxic alkaloids in larkspurs undergo flip-flop kinetics, meaning the rate of absorption of the alkaloids is slower than the rate of elimination. The implications of flip-flop kinetics in treating animals poisoned by larkspur is discussed.

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1. Introduction

There are over 60 species of larkspur plants (*Delphinium* spp.) in western North America (Burrows and Tyrl, 2013; Kingsbury, 1964; Knight and Walter, 2001). Larkspurs are acutely toxic to cattle, causing a significant number of cattle death losses every year (Nielsen and Ralphs, 1988; Pfister et al., 2002, 2003). There are three types of larkspurs categorized primarily by mature plant height and distribution as tall, low, and plains larkspurs (Pfister et al., 1999). The toxicity of all larkspurs is due to norditerpenoid alkaloids, which consist of two predominant types, the *N*-(methylsuccinimido) anthranoyllycoctonine (MSAL)–type including methyllycaconitine (MLA) and the non MSAL-type including the 7, 8-methylenedioxylycoctonine (MDL)-type such as the alkaloid deltaline (Panter et al., 2002; Pfister et al., 1999).

Previous studies have characterized the serum toxicokinetics of MLA and deltaline in cattle after oral dosing with several species of tall larkspurs (Green et al., 2009b, 2011) and low larkspurs (Green et al., 2012, 2013). In those studies, the elimination half-life of MLA was determined to be approximately 18–21 h and 12–16 h in tall and low larkspurs, respectively. However, when MLA was dosed IV to mice, the elimination half-life was calculated to be 12–18 min (Stegelmeier et al., 2003; Welch et al., 2008). One obvious reason for this discrepancy could be species differences, as highlighted by a recent study that demonstrated small species differences in the elimination half-life of MLA between cattle, sheep, and goats (Welch et al., 2016). However, a study was performed in rats wherein the elimination half-life of MLA was 19 min after IV dosing and 408 min after oral dosing (Turek et al., 1995), demonstrating that there was a clear effect of absorption on the kinetic profile of MLA in the same species. These observations have led us to hypothesize that larkspur alkaloids follow “flip-flop” kinetics, where the rate of absorption is slower than the rate of elimination. In a traditional serum concentration-time curve for an orally dosed compound, the terminal phase of the curve represents the elimination rate for the compound. However, in a flip-flop kinetic scenario the terminal phase is instead actually a reflection of the absorption rate due to the fact that the absorption rate of the compound is much slower than the elimination rate (Shargel and Yu, 1993). Although not a common scenario, flip-flop kinetics occur often enough to be discussed in chapters on toxicokinetics in

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general toxicology texts (Roberts and Renwick, 2014; Shen, 2013). The only way to ensure that the terminal phase of a serum concentration-time curve for a compound is indeed the elimination rate for a compound, is to dose the compound both orally and intravenously and compare the rates of the terminal phases. For most compounds the rates will be the same and thus the terminal phase of the oral concentration-time curve is the elimination rate. However, if the elimination rate is faster in the IV dosed animals, then flip-flop kinetics has occurred. Therefore, the objective of this study was to compare the serum toxicokinetic profiles of larkspur alkaloids when administered to goats orally and IV, in order to determine if larkspur alkaloids follow a flip-flop kinetic profile.

2. Materials and methods

2.1. Plant

Delphinium barbeyi was collected in the early flowering stage during July 2007 near Cedar City, Utah (N 37° 40.223′, W 112° 49.335′, at an elevation of approximately 3300 m; Poisonous Plant Research Laboratory collection 07-06). The plant material was air-dried, and ground to pass through a 2.4 mm mesh using a Gehl Mix-All model 55 (Gehl Company, West Bend, WI, USA). After processing, the ground plant material was stored in plastic bags away from direct light at ambient temperature in an enclosed shed until use. The norditerpenoid alkaloids in the plant material are stable under these conditions. The plant material was analyzed to obtain a mass spectrum of the alkaloid content, using a mass spectrometry method previously described (Gardner et al., 1999). Positive electrospray ionization mass spectra of alkaloid extracts indicated that deltaline (m/z = 508) was the major non MSAL alkaloid with MLA (m/z = 683) as the major MSAL alkaloid (Fig. 1). The plant material was analyzed for total norditerpenoid alkaloid content and MSAL-type alkaloid content using a Fourier-transform infrared spectroscopy (FTIR) method previously described (Gardner et al., 1997). This method measures the concentration of MSAL-type alkaloids and the concentration of total norditerpenoid alkaloids. Consequently, the concentrations of the non MSAL-type alkaloids were calculated by subtracting the concentration of MSAL-type alkaloids from the concentration of total norditerpenoid alkaloids. This collection of *D. barbeyi* contained 19.1 mg/g of total alkaloids with 9.6 mg/g of MSAL-type alkaloids. Thus the plant had a 1:1 ratio of non MSAL-to MSAL-type alkaloids.

A total alkaloid extract was obtained from the plant material following previously published methods (Pelletier et al., 1981; Pelletier S.W. et al., 1989; Manners et al., 1992). The alkaloid extract was suspended in physiological buffered saline (PBS) solution, and the pH was lowered with hydrochloric acid (1 N) to achieve solubility. Sodium hydroxide (1 N) was then added to the solution to raise the pH to near physiological pH, while still retaining solubility. The solutions (16 mg total alkaloid/mL) were stored in polypropylene falcon tubes at 4 °C overnight.

2.2. Animals

All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee. Eight Spanish goats weighing 22 ± 2 kg were maintained on alfalfa hay in their normal outdoor paddocks. The goats were randomly separated into two groups of four animals each. A single dose of the total alkaloid extract (4 mg total alkaloid/kg BW) was administered via oral gavage in 5–6 mL of phosphate buffered saline (PBS) or IV in 5–6 mL of PBS. While the oral gavage tube was still in the animal, it was flushed with approximately 50 mL of tap water to ensure none of the sample remained in the gavage tube. Blood was collected via jugular venipuncture at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 17, 24, 29, 34, and 48 h after oral dosing and at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h after IV dosing. Serum was separated from red blood cells by centrifugation and stored frozen at −20 °C.

2.3. Serum alkaloid analyses

The serum was analyzed for MLA and deltaline similar to that described previously (Welch et al., 2015) with the addition of parameters to quantitate deltaline. Matrix-matched standards were prepared as follows: a stock solution of MLA and deltaline was prepared at 1.0 mg/mL in ethanol and then 0.080 mL of each were diluted into 0.840 mL ethanol to provide a standard solution containing 80 μg/mL of each alkaloid. A 0.050 mL aliquot from the 80 μg/mL solution was added to 1.950 mL of sera from a non-treated goat and serially diluted with goat sera to give matrix standards in a range of 2000–1.9 ng/mL MLA and deltaline. From each time-course collected sera sample, and the matrix standards, a 0.500 mL aliquot was taken and placed in a 1.5 mL Eppendorf tube. An equal volume of acetonitrile (0.500 mL) was added to each sample. Samples were vortexed for 10–15 s and then centrifuged at 16,000 × g for 10 min. A 0.75 mL aliquot was then removed to a 1.5 mL autosample vial for analysis.

For the analysis, a Velos Pro LTQ (Thermo Scientific, San Jose, CA) mass spectrometer coupled with Agilent 1260 autosampler and Binary pump (Agilent Technologies, Santa Clara, CA) was used in-line with a Betasil C18 column (100 × 2.1 mm, 5 μm particle size; Thermo Scientific), with a guard column (10 × 2.1 mm) of equivalent phase. The column was eluted with a binary solvent gradient using 0.1% formic acid (solvent A) and acetonitrile (solvent B), at a flow rate of 0.400 mL/min and the following gradient mixture with time: 5%–15% B (0–1 min); 15–75% B (1–8 min); 75% B (8–10 min); 75-5% B (10–11 min); 5% B (11–16 min). The flow from the column was connected to a heated electrospray ion source. The mass spectrometer was set to scan selected positive ion MS/MS experiments during the following time segments: (0–4.5 min) deltaline parent ion m/z 508.3 with collision-induced dissociation (CID) at 28% power and (4.5–5.5 min) MLA parent ion m/z 683.3 with CID at 35% power. Reconstructed ion chromatograms used the following

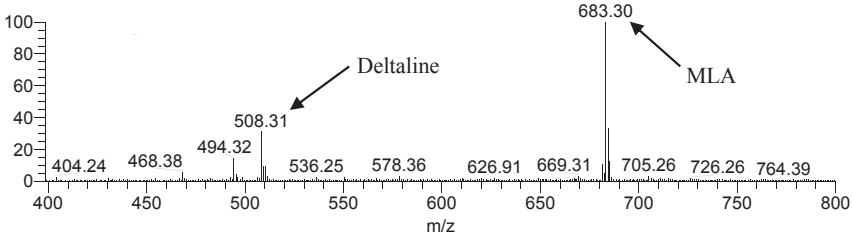

Fig. 1. Representative (+) esi-mass spectrum of the *Delphinium barbeyi* total alkaloid extract used in this study. Specific noted ions include deltaline (MH^+ m/z, 508) and methyllycaconitine (MH^+ m/z, 683).