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Studies on the teratogenicity of anabasine in a rat model

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ABSTRACT

A number of plant toxins have been shown to be teratogenic to livestock. The teratogenic action of some of these alkaloids is mediated by nicotinic acetylcholine receptors (nAChR). However, for many of these alkaloids it is difficult to obtain sufficient quantities of individual alkaloids to perform teratology studies in livestock species. Therefore the objective of this study was to determine if a rat model can be utilized to characterize the teratogenic nature of individual plant toxins that are nAChR agonists. In this study, we evaluated the teratogenicity of anabasine by feeding pregnant rats anabasine-containing rodent chow from gestational day (GD) 6–21. On GD21, the dams were euthanized and the gravid uteri were removed. The gravid uteri and individual pups were weighed. The pups were evaluated for bone malformations including cleft palate and scoliosis. Overall, the results of this study suggest that the rat is not a good model to study the teratogenicity of plant toxins that are nAChR agonists. It is possible that in the rat model, anabasine administered orally via the chow may not result in sufficient reduction in fetal movement to cause the significant malformations observed in livestock species.

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1. Introduction

A number of plant toxins have been shown to be teratogenic to livestock (Panter et al., 2011). The maternal consumption of plants that contain piperidine, pyridine, or quinolizidine alkaloids has the potential to cause developmental defects in animals (Green et al., 2013a). Many of the actions of these alkaloids are mediated by nicotinic acetylcholine receptors (nAChR). In the developing fetus, teratogenic piperidine alkaloid-mediated desensitization of fetal muscle-type nAChR is postulated to inhibit fetal movement, resulting in skeletal flexure defects and cleft palate (Green et al., 2013b; Panter et al., 1999). The inhibition of fetal movement disrupts the normal developmental process to cause multiple congenital contracture-type (MCC-type) deformities (arthrogryposis, kyphosis, lordosis, scoliosis, and torticollis) and cleft palate (Panter and Keeler, 1992; Weinzweig et al., 2008). The inhibition of fetal movement is thought to cause skeletal malformations through sustained alignment and positioning of the embryo/fetus *in utero* (Panter et al., 1990a, 1999).

The association between the inhibition of fetal movement by plant alkaloids and the formation of MCC-type defects is well-documented in livestock (Green et al., 2013e; Panter et al., 1999). When plants, such as lupine (*Lupinus* spp.), tobacco (*Nicotiana* spp.), or poison hemlock (*Conium* spp.), are consumed by pregnant females at the sensitive stage of development, the teratogenic alkaloids from the plants are believed to cross the placenta into the fetal compartment, to act at fetal muscle-type nAChR, and inhibit fetal movement. This has been documented with ultrasound imaging studies of fetuses in pregnant livestock dosed i.v. with the piperidine alkaloid coniine, or oral

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dosing studies with poison hemlock (*Conium maculatum*) and tree tobacco (*Nicotiana glauca*) (Panter et al., 1999; Panter and Keeler, 1992; Panter et al., 1990b).

There are numerous follow-up studies that need to be conducted to further characterize the teratogenic nature of the individual piperidine, pyridine, and quinolizidine alkaloids. Many of these alkaloids are enantiomers, and characterization of the differences in teratogenicity of the individual enantiomers is also needed. In this regard, studies have documented the stereoselective inhibition of fetal movement and the formation of MCC-type defects and cleft palates (Green et al., 2013b; Panter et al., 1990a; Panter and Keeler, 1992, 1993). However, many of these alkaloids are present in the plants as a mixture, and some of the alkaloids are not very abundant. Thus it is difficult to obtain sufficient quantities of many of these alkaloids to perform teratology studies in livestock species. Therefore, a small rodent model to study the teratogenicity of these alkaloids, including their enantiomers, would be valuable for investigating alkaloids present in only limited quantities. Consequently, the objective of this study was to determine if a rat model can be utilized to study and characterize the teratogenic nature of individual plant toxins that are nAChR agonists.

2. Materials and methods

2.1. Animals

Male and female Wistar rats (8 weeks old) were purchased from Simonsen Laboratories Inc., Gilroy, CA. Rats were acclimated for 3–4 d with free access to a commercially pelleted rodent chow (Harlan Teklad rodent diet (w) 8604) and tap water before beginning experiments. Rats were housed under controlled temperature (20–22 °C) and humidity, in a 12:12 h light:dark cycle. Rats were hand mated (2 females and 1 male per cage) for 12 h each night. Females were evaluated the following morning for the presence of a copulatory (mucus) plug and for the presence of sperm in the vaginal area as evidence of mating. The day sperm in the vaginal area were detected was considered gestational day (GD) 0. The pregnant rats were housed individually and dosed as outlined below beginning on GD6. All procedures were conducted under veterinary supervision and were approved by the Utah State University Institutional Animal Care and Use Committee.

Diets for the treated groups were prepared using the same commercially pelleted rodent chow (Harlan Teklad rodent diet (w) 8604), which was ground and mixed with anabasine and 10% corn starch in hot water to obtain 0, 50, 125, 250, 500, and 1000 μg anabasine/g rat chow. Anabasine was extracted from *N. glauca* following previously published methods (Keeler et al., 1984). The anabasine used for this study has been shown to be greater than 95% pure by gas chromatography-flame ionization detectors (GC-FID) (Welch et al., 2013a). Pellets, approximately 1 × 3 cm in size, were formed and dried overnight in an oven at 37 °C. The rat chow for the control group was prepared in the same manner, but with 90% commercial rodent chow and 10% corn starch.

Chow consumption and animal body weight were measured every two days beginning on GD6 through GD21. On GD21 the rats were euthanized by CO_2 asphyxiation and the ovaries and uteri were removed by cesarean section. The gravid uterus was weighed. The fetuses were removed from the uterus, dried of amniotic fluid and individually weighed. The number of implantation sites and resorptions was recorded. After being weighed, the fetuses were incubated in acetone for a minimum of 24 h, and subsequently eviscerated. For examination of the skeleton, the fetuses were submersed in a solution of 0.8% potassium hydroxide with alizarin-red S, which was changed daily for 3–5 days (Medeiros et al., 2008; Staples and Schnell, 1964). The fetuses were then stored in a solution of 40% ethyl alcohol, 40% glycerin, and 20% benzyl alcohol. Fetuses were visually evaluated, using a dissecting microscope, for any bone malformations including cleft palate and scoliosis.

2.2. Ultrasound protocol

Rats were restrained using common handling techniques by grasping the rat firmly around the neck and back and held such that their abdomen was facing up. The rats were examined transabdominally using an Aloka SSD-900V scanner fitted with a 7.5 MHz convex electronic thumb transducer (Wallingford, CT). Qualitative assessments of fetal movement were made via ultrasound on GD7, GD14, and immediately prior to euthanasia on GD21.

2.3. Analysis and statistics

All statistical analyses were performed using SigmaPlot (version 12.5, SPSS Inc., Richmond, CA). Statistical comparisons between multiple groups were performed using ANOVA with a Bonferroni posthoc test of significance between individual groups. Statistical comparisons of incidence rates between two groups were performed using a two-sided Fisher's exact test, using a 2 × 2 contingency table. Correlations between two factors were determined using Pearson Product Moment Correlation. Differences were considered significant at $P < 0.05$.

3. Results

Rats in the treated groups were fed rodent chow that contained anabasine from GD6-21. Rats fed rodent chow that contained 125 μg anabasine/g of chow, or greater,

Table 1
Feed consumption, body weight, and anabasine dose.

Group	*n*	Chow consumed[a] (g)		Body weight[b] (kg)		Anabasine dose[c] (mg/kg)	
		AVG	SD	AVG	SD	AVG	SD
CNT	15	279	27	0.32	0.02	0	0
50	5	295	4	0.34	0.02	43	2
125	6	245*	42	0.28	0.06	110	11
250	6	200*	16	0.30	0.03	168	20
500	10	97*	14	0.20*	0.03	247	35
1000	6	70*	11	0.15*	0.03	485	38

*Denotes difference from control group ($p < 0.05$).
[a] The amount of rodent chow consumed from GD6-21.
[b] Body weight at the time of necropsy on GD21.
[c] The total dose of anabasine received from GD6-21.