Effects of Dexamethasone on the Metabolism of Ergot Alkaloids in Endophyte-infected Fescue Seed Diet in Rat Liver Microsomal Cytochrome P450

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Story in Brief

Animals ingesting ergot alkaloids found in tall fescue grass display a variety of toxicological conditions. Dexamethasone (DXM) is a synthetic glucocorticoid that has been shown to induce enzymatic degradation of ergot alkaloids in liver microsomes. The objective of this study was to determine if DXM induces catabolism or clearance of ergot alkaloids in diets containing such compounds in endophyte-infected tall fescue seed. Sprague-Dawley rats (n = 36; ~ 250 g BW) were randomly assigned to 1 of 6 treatments arranged in a 2 x 3 factorial design. Main effects were diets containing 50% endophyte-infected ground tall fescue seed or 50% uninfected ground seed (3 vs 0 mg/kg of ergovaline and ergotamine) and DXM at 0, 30, and 60 micrograms/kg BW spread equally over 3 days. Intraperitoneal injections of corn oil carrier (with and without DXM) were given to all rats on d 15, 16, and 17. On d 21, rats were given anesthesia, exsanguinated, and eviscerated. Livers were weighed and microsomes prepared from liver tissue. Microsomes were assayed for metabolism of ergotamine and its isomer using high-performance liquid chromatography (HPLC) methodology. Body weight gain and ADG were decreased (P < 0.01) by increasing doses of DXM. Gross liver weight was affected (P < 0.01) by an interaction of DXM and diet. Infected seed diets increased (P < 0.05) the metabolism of ergotamine and ergotamine isomer across DXM concentrations. Comparison of amounts of P450 proteins by immunoassay suggests induction is affected by diet and DXM (P < 0.05), and that this influences metabolic activity in vitro.

Introduction

The symbiosis between tall fescue grass (Festuca arundinacea Schreb.) and endophytic fungus (Neotyphodium coenophialum) is responsible for drought and pest resistance and is related to production by the fungus of a variety of ergot alkaloids (Porter, 1995; West, 2000). These ergot alkaloids have a wide variety of physiological effects when ingested in small quantities, most of them detrimental to commercial agriculture. These effects are greatest at environmental extremes, and can prove deadly in either the hottest part of summer or the coldest part of winter. Beef cattle grazing infected fescue may gain half as much as those grazing non-infected, and they may have one-third to one-half lower pregnancy rates.

A wide variety of management practices have been explored to prevent the toxic effects of grazing fescue grass. Under common pasture conditions, ergovaline concentrations can range from 2 to 6 µg/g (Porter, 1995). Due to the variety of similar compounds under suspicion, it is difficult to relate precise symptoms to precise alkaloids. Animals have evolved a variety of methods of coping with the chemical defense systems that plants use to discourage predation (Cheeke, 1995). Unavoidable toxins, like ergot alkaloids, must be detoxified by other means. Based on the known metabolic pathways of ergot alkaloids, the P450 CYP3A4 enzyme is the human isoform responsible for their breakdown. Similar genes are evident in rats [CYP3A23] and cattle [CYP3A28]. In beef cattle, ergotamine undergoes a sequence of hydroxylations to four metabolites and their isomers, with similar activity occurring in rats (Moubarak and Rosenkrans, 2000).

This study was conducted to monitor other physiological changes and to characterize the dose-dependent responses in metabolism and catabolism of ergotamine to dexamethasone and dietary ergot alkaloids, and to see if diets containing ergot alkaloids modify dexamethasone induced P450 metabolism of alkaloids.

Experimental Procedures

Dexamethasone, ergotamine, nicotinamide adenine dinucleotide phosphate (NADP+), D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and other chemicals were obtained from Sigma-Aldrich, St. Louis, Mo. Experimental animals were from Harlan Teklad, Madison, Wis. Metabolite analysis was performed using a high-performance liquid chromatography (HPLC) system from Gilson Inc., Middleton, Wis. Monoclonal antibodies were obtained from GENTEST Corporation, Woburn, Mass. Six-week old male rats (n = 36, ~250 g body weight) were housed in the small animal facility at University of Arkansas. Water and formulated diets were available ad libitum. Temperature was maintained at 24°C (76°F) and lights were on 18 hours and off 6 to simulate summertime conditions. Dietary treatment was pelleted food containing 50 percent Harlan Teklad lab chow and either 50 percent endophyte infected ground seed or noninfected ground fescue grass seed. Ergot alkaloids ergovaline and ergotamine in this diet were assayed by HPLC to be 0 and 3 ppm by the method of Moubarak et al. (1996). After 14 days of acclimation to cages and fescue diet the dexamethasone (DXM) treatment was administered. Dexamethasone treatments were doses of 0, 30, or 60 µg drug per kg body weight. This was divided into 3 injections on days 15, 16, and 17 of the study. Per animal dose was calibrated by variable injection of 100 µg/cc dexamethasone suspended in corn oil. Animal weights and feed consumption were monitored for 1 week, at which point animals were sacrificed. Livers were frozen, and then

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prepared by the method of Kremers et al. (1981). Sodium phosphate was omitted to maintain microsome activity. During the preparation, frozen liver tissue was ground at 1 g per 5 ml of buffer (250 mM sucrose, 100 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4,) with Tissue-Tearor homogenizer. This slurry was then centrifuged at 800 g for 10 min. The pellet was discarded, and supernant centrifuged at 13,500 g for 20 min. The supernatant from this was then spun at 105,000 g for 1 hour. The pellet was then suspended in storage buffer (100 mM sodium phosphate, 20% glycerol) and frozen in 50 µl aliquots. Microsome activity assay was performed in cofactor generating system by the method of Peyronneau et al. (1994). Microsomal protein (0.5 mg) was suspended in 500 µl cofactor generating buffer (100 mM Tris-HCl pH 7.5, 0.1 mM EDTA, 26 mM NADP+, 66 mM D-glucose-6-phosphate, 66 mM magnesium chloride, 1 U glucose-6-phosphate dehydrogenase in sodium citrate) along with 1 µg ergotamine. After 30 min, activity was stopped using concentrated acetic acid, and samples were centrifuged to remove particulate contaminants.

Metabolic activity of the microsomes was tracked by the conversion of ergotamine and its isomer into various metabolites. Separation was performed using a Gilson 714 HPLC system with gradients of acetonitrile and 2.6 mM ammonium carbonate in 10% methanol over a 3 * 3 CR C18 column. Detection was performed with Gilson 121 fluorescence detector with excitation at 250 nm and emission at 370 long pass filter (Moubarak and Rosenkraus, 2000).

Liver microsome P450 concentrations were compared by dot-blot performed using standard procedures obtained from Bio-Rad protocols and GENTEST monoclonal antibody product instructions. Total microsomal protein was measured by method of Lowry et al. (1951) and equalized at 7 µg per sample. Samples were then loaded into vacuum filtration system and transferred to nitrocellulose. Primary antibody was GENTEST’s WB-MAB-3 against human P450 at 1:500 concentration, followed by secondary antibody of horseradish-peroxidase conjugated rabbit anti-mouse at 1:500 concentration. Nonspecific binding was blocked with 1% BSA. Color was developed with Sigma NBT-BCIP liquid substrate system, and analysis of color development was performed using Kodak 1D densitometry software.

Data were analyzed by PROC GLM of SAS (SAS Institute, Inc., Cary, NC) Models included dexamethasone, dietary ergot alkaloids, and the interaction between them. When the F test was significant (P < 0.10) the means were separated using the PDIFF option of GLM.

**Results and Discussion**

Body weight change was affected by presence of ergot alkaloids (P < 0.05) and by dexamethasone (P < 0.01); however there was no interaction between dietary alkaloids and DXM dosage for body weight change (Table 1; Figure 1). When averaged over the DXM treatments rats on the E- diet lost -0.95 ounces while those on the E+ diet lost significantly more (-1.65 ounces). Means by level of DXM were -0.05, -1.53, and -2.32 ounces for 0, 30, and 60 µg/kg of DXM, respectively. The linear dose response slopes based on DXM treatments were parallel indicating similar responses to the DXM treatment for both dietary treatment groups. Expressed as ADG, means were -0.008, -0.218, and -0.331 ounces for DXM levels of 0, 30, and 60 µg/kg, respectively, and -0.135 and -0.236 ounces for E- and E+ diets.

This change in weight may have been affected by differential feed consumption by experimental animals. Feed consumption was affected by both DXM and ergot alkaloids (P < 0.01; Table 1). Illustrated in Figure 2, average daily feed consumption showed a similar decline to that seen with weight gain with additional DXM. This is consistent with symptoms of fescue toxicosis, but not with DXM treatment.

There was an interaction between DXM dose and dietary ergot alkaloids (P < 0.01) for liver weight (Figure 3; Table 1); but when expressed as a ratio of body weight there was only a tendency for an interaction (P = 0.14). The ability of liver microsomes to modify substrate in vitro as measured by peak area concentrations (Figure 4) was affected by both ergot alkaloid diet and DXM treatment (P < 0.05), but there was no interaction. Substrate concentration represented by the mean peak area for ergotamine was 1,290,576 for rats on E- diets and 990,858 for rats on E+ diets. Mean peak areas for DXM levels of 0, 30, and 60 µg/kg were 1,044,422, 984,379, and 1,393,351, respectively, with the 30 DXM level being lower than the 60 DXM level (P < 0.05). The greatest decrease in ergotamine peak area is presumed to be in animals that had the most P450 activity. They received infected fescue seed in their diet and dexamethasone at 30 µg/kg. Ergotamine isomer metabolism (data not shown) was affected similarly to ergotamine.

Dot-blotting of microsomal protein measures expression of P450 using cross-reactivity to human P450. Optical analysis of the mean color development of each sample reveals a tendency for an interaction (P = 0.11) between dexamethasone and dietary ergot alkaloid resulting in reduction of expression of P450 due to dexamethasone in the E- diet and an increased expression of P450 in the E+ diet (Figure 5).

High doses of corticosteroids have been shown to greatly influence metabolism, as have dietary ergot alkaloids (Oliver et al., 2000). This study was most interested in interactions between the two drug types, especially to determine if dexamethasone could improve some of the deleterious effects of ergot alkaloids.

Dexamethasone is used to relieve stress because it stimulates gluconeogenesis from muscle breakdown and stimulates appetite, which can result in overall weight gain. This study demonstrated that feed consumption and body mass were decreased by dexamethasone treatment. This inconsistency may be due to the low dosages given in this study. One to 6 mg/kg therapeutic dose up to 400 mg/kg to induce P450 in rats have been reported in the literature. Doses given in this study were significantly lower, at total doses of 30 and 60 µg/kg. From a performance standpoint, the healthiest animals were those that received no treatment at all. Both ergot alkaloids and dexamethasone treatment independently reduced performance in a linear, dose dependent manner. This is apparent in weight change, feed consumption, and average daily gain. Liver weight and its relation to body weight would be impacted by both changes in blood volume due to vasoconstrictive effects of ergot and by increased P450 expression due to dexamethasone.

**Implications**

The presumption of this research was that treatment with dexamethasone would increase the metabolism of alkaloids via the induction of P450 enzymes that increase clearance. The implications of this research for animal health suggest that the increase in metabolism of ergot alkaloids by dosage with dexamethasone may not be the most efficient way of reducing the effects of fescue toxicosis on production and performance.
Table 1. Treatment means for effects of dexamethasone dosage with and without dietary ergot alkaloids.

<table>
<thead>
<tr>
<th>Item</th>
<th>No endophyte (E-)</th>
<th>Endophyte present (E+)</th>
<th>SE</th>
<th>Significant effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight change, ounce</td>
<td>0.317</td>
<td>-1.238</td>
<td>-1.919</td>
<td>-0.423</td>
</tr>
<tr>
<td>Total feed consumed, ounce</td>
<td>5.617</td>
<td>3.815</td>
<td>3.727</td>
<td>4.554</td>
</tr>
<tr>
<td>Average daily gain, ounce</td>
<td>0.045</td>
<td>-0.177</td>
<td>-0.274</td>
<td>-0.060</td>
</tr>
<tr>
<td>Liver weight, ounce</td>
<td>0.433</td>
<td>0.374</td>
<td>0.393</td>
<td>0.397</td>
</tr>
<tr>
<td>Liver mass:body mass ratio</td>
<td>0.041</td>
<td>0.038</td>
<td>0.041</td>
<td>0.031</td>
</tr>
<tr>
<td>Total microsomal protein (mg/ml)</td>
<td>14.113</td>
<td>12.212</td>
<td>10.725</td>
<td>14.835</td>
</tr>
</tbody>
</table>

E = endophyte effect ($P < 0.01$),
e = endophyte effect ($P < 0.05$),
D = dexamethasone effect ($P < 0.01$),
d = dexamethasone effect ($P < 0.05$),
D*E = dexamethasone by endophyte interaction ($P < 0.01$),
d*e = dexamethasone by endophyte interaction ($P = 0.14$).
Fig. 1. Body weight gain as affected by dietary ergot alkaloids (E- vs E+) and dexamethasone (DXM) treatments. There was both a DXM effect ($P < 0.01$) and an ergot alkaloid effect ($P < 0.05$) but no interaction. 

a, b, c DXM treatment means with no letter in common differ ($P < 0.01$).

Fig. 2. Average feed consumption as affected by dexamethasone (DXM) dose and dietary ergot alkaloids (E- vs E+). There was both an ergot alkaloid effect ($P < 0.05$) and a DXM effect ($P < 0.01$) but no interaction.

a, b DXM treatment means with no letters in common differ ($P < 0.01$).

Fig. 3. Liver weights as affected by dietary ergot alkaloids (E- vs E+) and dexamethasone (DXM) treatments. There was an interaction between ergot alkaloid diet and DXM treatment ($P < 0.01$).

a, b, c Treatment means with no letter in common differ ($P < 0.01$).
Fig. 4. Ergotamine peak area as affected by dietary ergot alkaloids (E- vs E+) and dexamethasone (DXM) dose. There was both an ergot alkaloid effect and a DXM effect ($P < 0.05$) but no interaction. a,b DXM treatment means with no letter in common differ ($P < 0.05$).

Fig. 5. Mean color development on P450 dot blot for ergot alkaloid (E- vs E+) and dexamethasone (DXM) treatments. There was a tendency ($P = 0.11$) for an interaction, but neither main effect was significant ($P > 0.50$).