Effect of endophyte infected tall fescue seed extract on cytochrome P450 system

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Story in Brief

Endophyte infected tall fescue is the base diet for nearly all beef cattle in the southern U.S. It has been linked to various toxicological conditions due to the presence of large numbers of ergot alkaloids. This study was designed to study the effects of endophyte infected tall fescue seed extract and selected ergot alkaloids on the detoxification pathway by cytochrome P450 enzyme system. Tests were performed using the P450-Glo cytochrome P450 enzyme activity kit (Promega, Wis.), according to the manufacturer’s manual. Endophyte infected tall fescue seed was extracted with 50/50 methanol/25 mM ammonium carbonate, cleaned and concentrated on Strata-X reversed phase column (Phenomenex). The extracts were evaluated on an HPLC, and then tested using a serial dilution method. Commercially available ergonovine (EN) and ergocryptine (EC) were tested individually using 0 to 44 nM concentrations. Seed extract of endophyte infected tall fescue produced a significant (P < 0.05) dose dependent inhibition of cytochrome P450 enzyme activity similar to that produced by the commercially available ergot alkaloids. Ergocryptine and ergonovine inhibited cytochrome P450 enzyme activity in a dose dependent manner (P < 0.05) with ergocryptine being most potent, followed by ergonovine (70% and 10% at 44 nM concentration). The similarity of the inhibition curves of seed extract to that of the commercially available ergot alkaloids suggest a related mode of action and that the use of such ergot alkaloids and cytochrome P450 assay is a good model to study the toxicity of tall fescue. Furthermore, it provides the foundation to identify the individual toxic components of purified endophyte infected tall fescue extract.

Introduction

Tall fescue grass is considered to be the primary diet for nearly all beef cattle in southern United States and has been linked to the incidence of fescue toxicosis syndrome due to the presence of ergot alkaloids. Cytochrome P450 (CYP) enzyme systems play a key role in the biotransformation of many endogenous and exogenous compounds including both toxins and drugs (Porter and Coon, 1991, Pollock, 1994). The CYP enzyme family consists of a large number of proteins with different substrate specificities and catalytic properties which are membrane-bound, mostly localized to the endoplasmic reticulum and in mitochondrial inner membranes. Ergot alkaloids found in tall fescue are a large family of chemical compounds with two major subfamilies. The tetracyclic ergoline nucleus group, such as lysergic acid ethylamide, ergonovine, and lysergic acid, and the ergopeptide alkaloids group which share the tetracyclic ergoline nucleus in addition to tricyclic amino acids such as ergotamine, ergocryptine, and bromocriptine. The metabolism of ergot alkaloids, such as bromocriptine, dihydroergotamine, and other structurally similar ergot derivatives is mediated mainly by CYP3A4 (Ball et al., 1992). Moochhala et al. (1989) reported that bromocriptine interferes with P450-dependent oxidative metabolism of xenobiotics. Later it was demonstrated that cytochrome P450 3A exhibits a particularly high affinity for ergopeptides. Ergot alkaloids have been shown to be metabolized by CYP3A; however, information on the effects of this alkaloids on CYP3A activates is very limited. The metabolism of ergot alkaloids has been linked to CYP3A; therefore, activation or inhibition of the induction process of such enzyme systems can have severe consequences on the metabolism of the ergot alkaloids. Witkamp et al. (1995) reported that tiamulin, a semisynthetic antibiotic frequently used in agricultural animals, strongly inhibited the hydroxylation rate of testosterone at the 6 beta position via the formation of a cytochrome P450 3A4 metabolic intermediate complex in both microsomes and hepatocytes. Although we have reported previously (Moubarak et al. 2003) the lack of an induction effects of dihydroergotamine and ergonovine on rat CYP3A, this study was designed to examine the effects of seed extract of endophyte infected tall fescue and selected ergot alkaloids on the detoxification pathway by cytochrome P450 (CYP3A4) enzyme system in vitro.

Materials and Methods

All the chemicals and reagents used in these experiments were of the highest quality available and were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless stated otherwise. Tests were performed using the P450-Glo CYP3A4 enzyme activity kit (Promega, Wis.), according to the manufacturer’s manual. Briefly, 12.5 µl of test compound (seed extract or pure pushed ergot alkaloids) was added per well of a 96-well plate, followed by 12.5 µl of the 4 times concentrated CYP450 reaction mixture with and without CYP450 for the blank reactions. Then the plate was pre-incubated at 37 °C for 10 minutes. The reaction was initiated by adding 25 µl of the 2 times concentrated NADPH regeneration system and after 30 minutes incubation at 37 °C, 50 µl of reconstituted luciferin detection reagent was added. The plate was mixed for 10 seconds on an orbital shaker and incubated at room temperature for 20 minutes to stabilize the luminescent signal. And then the luminescence was recorded using Perkin Elmer 1420 Victor 3 V.

Endophyte infected tall fescue seed (E+) was extracted with 50/50 methanol/25 mM ammonium carbonate (10 ml/gm) for one hour, cleaned and concentrated on Strata-X reversed phase column (Phenomenex). The extracts were evaluated by a Millennium-32 Workstation HPLC system with auto-sample injector and a gradient programmer. The detection was accomplished using a Applied Biosystems 980 programmable fluorescence detector (excitation at 250 nm and emission at 370 nm long pass filter). Separation was conducted on a 3 × 3 CR C18 cartridge column using acetoniitrile and 2.6 mM ammonium carbonate in 10% methanol gradient elution at 1 ml/min flow rate. The extracts were evaluated using 80% serial dilution method, and the commercially available ergonovine (EN) and ergocryptine (EC) were tested individually using 0 to 44 nM concentrations.

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Results and Discussion

Toxins found in seed extract of endophyte infected tall fescue (Fig. 1) influenced CYP3A4 enzyme activity in a similar inhibition action as that of commercially available ergot alkaloids (EN and EC). Seed extract of E+ produced a ($P < 0.05$) dose dependent inhibition (59 and 81% inhibition at 11 and 100% of extract concentrations respectively) of CYP3A4 enzyme activity. Non-infected tall fescue seed extract (E-) produced no significant effect on CYP3A4 activity due to the lack of the toxins (Fig. 3). Ergocryptine and EN inhibited CYP3A4 enzyme activity in a significant ($P < 0.05$) dose dependent manner (0.0 to 44.0 nM) with EC being most potent (70%), and EN the least (10%) at 44 nM concentrations (Fig. 2). The similarity of the inhibition curves and the chemical structure of toxins found in seed extract to that of the commercially available ergot alkaloids EC and EN suggest that the toxins found in E+ appears to target the same location on the CYP3A4 and follow the same mechanism of inhibition.

Implications

The connection between the effects of fescue toxins and specific effects of the ergot alkaloids, ergocryptine and ergonovine, on CYP enzyme systems has been established. Such association helps in understanding of the effects of E+ in general and lays the foundation to study the detailed effects of each single component of fescue toxins on liver detoxification mechanisms. Also the use of P450-Glo CYP3A4 enzyme activity kit appeared to be a good instrument to study and identify the toxic individual components of purified endophyte infected tall fescue extract.

Literature Cited


Fig. 1. High-performance (pressure) liquid chromatography chromatogram showing the typical profile of seed extract of endophyte infected tall fescue showing lysergic acid amide group (LAA), ergonovine (EN), ergovaline and the isomers (EV and EV iso), ergosine (ES) and ergotamine (ET).
Fig. 2. Effects of increasing concentrations of ergonovine (EN) and ergocryptine (EC) on the in vitro activity of CYP3A4.

Fig. 3. Effects of increasing concentrations of seed extract of endophyte infected tall fescue on the in vitro activity of CYP3A4.