Interaction of Fescue Toxin with Liver Enzyme P450 3A

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Story in Brief

This study was conducted to investigate the effect of the ergot alkaloid, ergotamine (ET), on the induction of cytochrome P450 3A. Sprague-Dawley rats were treated by injecting intraperitoneally as follows: control (0.5 ml of only corn oil for 4 days); dexamethasone treatment (100 mg/kg of BW of dexamethasone in corn oil for 1, 2, 3 or 4 days); and ergotamine treatment (100 mg/kg of BW of ergotamine in corn oil for 4 days). Liver tissues were collected from each group (n = 5/treatment, total of 30) and liver microsomes prepared. Cytochrome P450 3A activity was evaluated using ET and its isomer as substrates in medium containing liver microsomes and NADPH (nicotinamide adenine dinucleotide) at 37°C for 30 min. The disappearance of substrate and the appearance of metabolites were measured using high pressure liquid chromotagraphy (HPLC). Microsomes from rats treated with dexamethasone were 5 times more ($P < 0.01$) active than microsomes from control animals in the biotransformation of ET (32.1 and 7.0 nM/min/mg protein, respectively; SE = 4.83) or ET isomer (21.6 and 4.7 nM/min/mg protein, respectively SE = 1.71) into corresponding metabolites. The amount of substrate (ET or ET isomer) remaining after incubation with cytochrome P450 3A did not differ ($P > 0.05$) between ergotamine-treated and control rats (5.2 vs. 7.0 nM ET/min/mg protein; SE = 4.83, or 1.5 vs. 4.7 nM ET isomer/min/mg protein; SE = 1.70). When ketoconazole was used as a specific inhibitor of cytochrome P450 3A, ergotamine metabolisms were inhibited in a dose dependent fashion. Data presented in this study suggest that although ET and its isomer are ideal substrates for the isozyme cytochrome P450 3A, they have no effect on the induction of cytochrome P430 3A after 4 days of treatment.

Introduction

When the subject is fescue toxicity, nothing is more important than the ability of the liver to detoxify those toxins. Cytochrome P450 (CYP) is an inducible enzyme system that plays a key role in the detoxification and clearance of many compounds including both toxins and drugs (Pollock, 1994). The CYP 1-3 families are active in the metabolism of xenobiotics with CYP 3A subfamilies being the most important in drug metabolism (Wright and Paine, 1994). The metabolism of ergot alkaloids, such as bromocriptine, ergotamine, and other structurally similar ergot derivatives, is mediated mainly by CYP 3A4. Moochhala et al. (1989) reported that bromocriptine interferes with P450-dependent oxidative metabolism of xenobiotics. Later it was demonstrated that CYP 3A exhibits a particularly high affinity for ergopeptides. Activation or inhibition of the induction process of such enzyme systems can have severe consequences. Witkamp et al. (1995) reported that tiamulin, a semi-synthetic antibiotic frequently used in agricultural animals, strongly inhibited the hydroxylation rate of testosterone at the 6 beta position via the formation of a CYP 3A4 metabolic intermediate complex in both microsomes and hepatocytes. This report was designed to study the effects of administering ergotamine (ET) in vivo on the induction of the CYP 3A family using dexamethasone as a specific inducer for similarity and to evaluate the in vitro interaction of ET with CYP 3A using ketoconazole as a specific inhibitor.

Experimental Procedures

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. Sprague-Dawley rats (n = 30; BW ~250 g) were allowed ab libitum access to water and chow. Rats were randomly assigned to treatment and treated intraperitoneally with one of the following treatments: 1) Control treatment (n = 5 rats, injections of 0.5 ml of corn oil for 4 consecutive days), 2) dexamethasone (DXM) treatment (n = 20 rats, injections of 100 mg/kg of BW of dexamethasone in 0.5 ml of corn oil for 1, 2, 3 or 4 consecutive days) and 3) ET treatment (n =5 rats, injections of 100 mg/kg of BW of ergotamine in 0.5 ml of corn oil for 4 consecutive days). Approximately 24 h after the last injection, each rat was anesthetized with chloroform, decapitated, and the liver was harvested. Livers were stored at –20°C until microsomes were prepared.

Michaelis-Menten kinetics were used to evaluate the interaction of CYP 3A with ET, and the linearity of the Lineweaver-Burk plot of 1/V (velocity) versus 1/S (substrate) was further examined by measuring enzyme activity at various concentrations (2.0 to 20.0 μM) of the enzyme substrate, ET, in the presence and absence of kentoconazole (3.0 and 5.0 μM).

Liver microsomes were prepared as reported by Moubarak and Rosenkrans (2000). Briefly, liver tissues (3 to 5 g) were diced with scissors and then washed with 150 mM sodium chloride buffer. The diced tissue was ground (1 g tissue/10 ml of buffer (250 mM sucrose, 100 mM Tris-HCl, 1 mM EDTA (ethylenediaminetetracetic acid), pH 7.4)) with ice-cold medium using a precooled blender for 10 to 20 sec and further homogenized using a Polter-Elvehjem (5 X). The homogenate was sequentially centrifuged at 800 x g for 10 min, at 13,500 x g for 20 min collecting the supernatant and then at 105,000 x g for 60 min collecting the pellet which contained the microsomal fraction. The pellet (microsomal fraction) was resuspended in buffer containing 100 mM sodium phosphate and 20% volume/volume glycerol. The protein concentration after resuspension was approximately 40 mg/ml. Aliquots of

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microsomal suspensions were stored at –20°C and were used within 20 to 30 days. Protein concentration was determined by the method of Lowry using bovine serum albumin as the standard.

Ergotamine and its isomer (ET-iso) were used as substrates to assay presumptive CYP 3A4 activity in vitro. They were analyzed using a standard assay method in 330 µl of assay medium, 100 µl of cofactor generating system (nicotinamide adenine dinucleotide; NADPH), 20 µl of ET (final concentration was 4 µg/ml of fully isomerized ET), and 50 µl microsomal protein (final concentration was 0.4 mg/ml). The assay medium (pH 7.4) consisted of 100 mM potassium phosphate, 0.1 mM EDTA, and 5.0 mM $MgCl_2$. The NADPH generating system consisted of assay medium with 10 mM NADP+, 10 mM D-glucose-6-phosphate, and 2.0 U/ml of Glucose-6-phosphate dehydrogenase. The reactions were initiated by adding the NADPH generating system and were terminated after 30 min by adding 100 µl of 94% acetonitrile and 6% glacial acetic acid. After the stop solution was added, the mixture was centrifuged at 12,000 x g for 4 min. Supernatant (150 µl) from each reaction was examined for the disappearance of ET and ET-iso and the appearance of their metabolites using the high pressure liquid chromatography (HPLC) method described by Moubarak and Rosenkrans (2000).

Data for the dexamethasone treated rats were analyzed using one-way ANOVA with day as the independent variable and concentration of remaining substrate (ET or ET-iso) as the dependent variables. Induction data for the 3 treatments (Control, DXM, and ET) after 4 days of treatment were analyzed using one-way ANOVA with treatment as the independent variable and concentration of remaining substrate (ET or ET-iso) as the dependent variable. The kinetics data of kentoconazole inhibition were interpreted using the double reciprocal plot method of 1/S versus 1/V.

Results and Discussion

Data in Figure 1 represent the disappearance of ET and its isomer after incubation with liver microsomes from rats that had been treated sequentially for 1, 2, 3, or 4 days with 100 mg/kg of BW of dexamethasone, a specific inducer of cytochrome P450 3A. Dexamethasone induced cytochrome P450 3A activity in rats to a significant ($P < 0.01$) level only after the third and fourth days of treatments. These results indicate that the mechanism of DXM induction of rat liver microsomal cytochrome P450 3A was time dependent, and it took at least 3 to 4 successive days of treatments to reach the maximum level of activity. Liver microsomes from rats treated with dexamethasone were 5 times more ($P < 0.01$) active than microsomes from the control animals in the biotransformation of ET (32.1 vs. 7.0 nM/min/mg protein, respectively; SE = 4.83) or ET-isomer (21.6 vs. 4.7 nM/min/mg protein, respectively SE = 1.71) into their corresponding ET metabolites.

The induction of CYP 3A4 is mainly regulated by the novel orphan receptor or pregnane X receptor (PXR), but other receptors including the constitutively active receptor (CAR) and, indirectly, the glucocorticoid receptor (GR) are involved. Dexamethasone, one of the glucocorticoids, influences several aspects ofcytochrome P450 3A induction. However, most of these effects are not dependent on GR binding to CYP genes, but rather on complex protein-protein interplay between GR and various other receptors (Honkakoski and Negishi, 2000). Information on how or if ET fits into the induction processes of CYP 3A rat is limited or not available.

Ergotamine treatment for 4 consecutive days produced no significant ($P > 0.05$; 5.2 vs. 7.0 nM ET/min/mg protein; SE = 4.83, and1.5 vs. 4.7 nM ET-iso/min/mg protein; SE = 1.70) increase in the cytochrome P450 3A activity over that of the control animals (Fig. 2). Moubarak et al. (2003) showed that treatment of rats with similar ergot alkaloids, dihydroergotamine (DHET) or ergonovine (EN), at a concentration of 100 mM did not produce any significant increase in the cytochrome P450 3A activity over that of control rats. Although it is generally accepted that most compounds that are metabolized by CYP are to some degree inducers of that CYP enzyme system, data from this study and from a previous study in our laboratory have indicated that all of the ergot alkaloids studied (ET, DHET, and EN) have very small or no induction effects on CYP 3A in rats, yet those alkaloids have been shown to be metabolized by rat CYP 3A. Previous studies have demonstrated that both DXM and ET can also inhibit the in vitro metabolism of tacrolimus mediated by the CYP 3A subfamily (Christians et al., 1996). Another study showed that dihydroergocryptine, a dopamine agonist for the treatment of Parkinson's disease, has an inhibitory effect on CYP 3A4-mediated testosterone metabolism and additionally could induce CYP 3A4 and CYP2E1 mRNA when added at 10 µM to cultured human hepatocytes (Althaus, et al., 2000). Moubarak and Rosenkrans (2000) reported that both ergonovine and dihydroergotamine inhibited in vitro CYP 3A4 activity in a dose dependent manner when ET was used as a substrate, producing quadratic inhibition curves. One can ask, is it possible for a group of compounds such as the ergot alkaloid group (ET, DHET, EN, etc.) to play a double role? One role is to be involved at the upstream induction of CYP 3A and another is to directly interact with the structure of the enzyme inhibiting its catalytic activity. Ergot alkaloids have been demonstrated to affect the cytochrome P450 system, especially isoenzyme CYP 3A4, by binding to the isoenzyme as a substrate (Moubarak and Rosenkrans, 2000).

The linearity of the Lineweaver-Burk plot of 1/V (V_{max}), versus 1/S (ET and ET isomer) shows that the interaction between the substrate (ET) and cytochrome P450 3A in both the presence and absence of ketoconazole (Fig. 3) follows Michaelis-Menten kinetics. Furthermore, the fact that the slope did not change with the addition of ketoconazole, and the decrease in the velocity of the reaction (V_{max}), indicated an uncompetitive inhibition. Thus ketoconazole interacts with the enzyme-substrate complex.

In this study we have demonstrated that although both ET and ET-iso are ideal substrates for cytochrome P450 3A, they appear to have no induction effect on rat hepatic cytochrome P450 3A during the 4 days of treatment when compared to the classic dexamethasone induction. The data from the in vitro interaction among ET, cytochrome P450 3A and its specific inhibitor (ketoconazole) showed that the binding site for ET was different from that for ketoconazole in rat hepatic cytochrome P450 3A.

Acknowledgments

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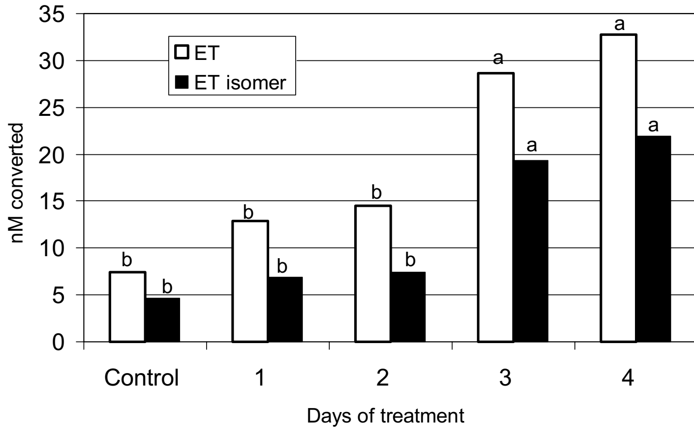

Fig. 1. The effects of intraperitoneal injections of dexamethasone (DXM) on the induction of rat liver cytochrome P450 3A activity over four consecutive days of injection. Means represented by bars containing the same letter (within ET or ET isomer) did not differ (P > 0.05).

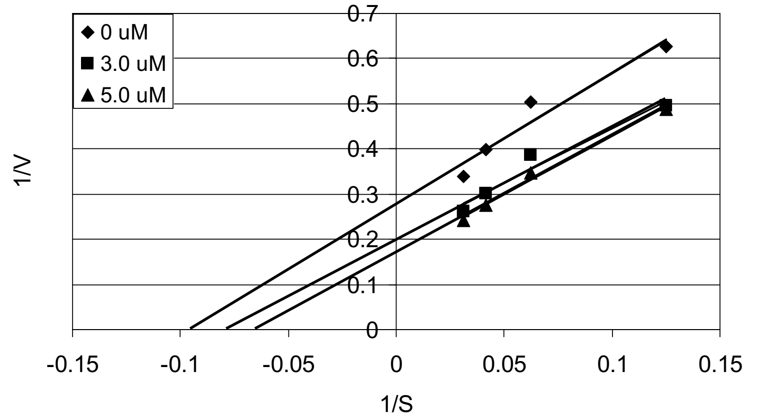

Fig. 3. Lineweaver-Burk plots of rat hepatic cytochrome P450 3A4 activity in 0, 3, and 5 uM of ketoconazole.

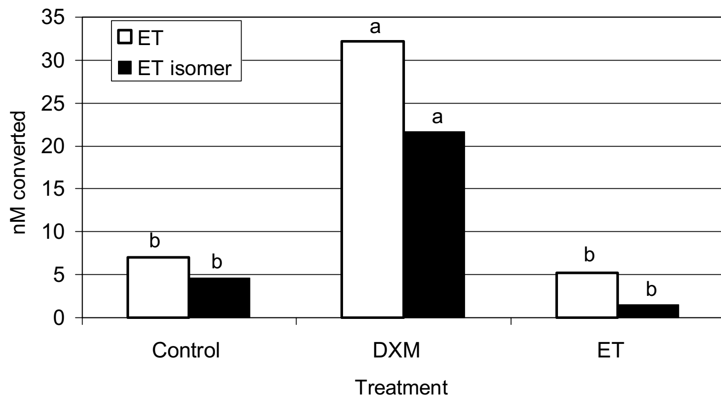

Fig. 2. The amount of substrate (ET or ET isomer) converted after incubation with rat hepatic cytochrome P450 3A preparation from control (CON), dexamethsone (DXM) and ergotamine (ET) treated rats. Means represented by bars containing the same letter (within ET or ET isomer) did not differ (P > 0.05).