Interaction of ergotamine with liver Cytochrome P450 3A in rats

Ali S. Moubarak*, Hehai Wang, Zelpha B. Johnson, Charles F. Rosenkranz

Department of Animal Science, University of Arkansas, Fayetteville, USA; *Corresponding Author: moubarak@uark.edu

Received 12 July 2012; revised 27 August 2012; accepted 15 September 2012

ABSTRACT

This study was conducted to investigate the effect of the ergot alkaloid, ergotamine (ET), on the induction of CYP3A and the interaction in vivo and in vitro with ET. Sprague-Dawley rats were treated intraperitoneally for 4 days as follows: control (injecting with 0.5 ml of only corn oil); dexamethasone treatment (injecting with 100 mg/kg of dexamethasone in corn oil); and ergotamine treatment (injecting with 100 mg/kg of ergotamine in corn oil). Liver tissues were collected from each group (n = 5, total of 30 rats) and liver microsomes were prepared. Cytochrome CYP3A activity was evaluated using ET and its isomer as substrates in medium containing liver microsomes and NADPH at 37˚C for 30 min. HPLC was used to measure the disappearance of the substrate and the appearance of the metabolites. Liver microsomes from rats pretreated with dexamethasone were five times more (P < 0.01) active than microsomes from the 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 = 1.7079) into its corresponding ET metabolites. The ergotamine treatment produced no increase (P > 0.05) in activity of CYP3A when compared to the control group (5.2 vs 7.0 nM ET/min/mg protein; SE = 4.83) or ET isomer (1.5 vs 4.7 nM ET isomer/min/mg protein; SE = 1.70). When ketoconazole was used as specific inhibitor of CYP3A, ergotamine metabolisms were inhibited in a dose dependent fashion reaching a maximum at an inhibitor to substrate ratio of greater than one and LD50 at 0.5 nM of ketoconazole/mg protein. The data presented in this study suggest that although the ergot alkaloids ergotamine and its isomer are ideal substrates for the isozyme CYP3A, these compounds have no effect on the induction of CYP3A after 4 days of treatment.

Keywords: Microsomes; Liver; Rat

1. INTRODUCTION

Cytochrome P450 (CYP) enzyme systems play a key role in the biotransformation of many endogenous and exogenous compounds including both toxins and drugs [1-4]. 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. CYP1-3 families are active in the metabolism of xenobiotics with CYP3A subfamilies being the most important in drug metabolism [5]. The metabolism of ergot alkaloids, such as bromocriptine, ergotamine and other structurally similar ergot derivatives is mediated mainly by CYP3A4 [6]. Moochhala et al. [7] reported that bromocriptine interferes with P450-dependent oxidative metabolism of xenobiotics. Later it was demonstrated that cytochrome P450 3A4 exhibits a particularly high affinity for ergopeptides. The CYP3A family expression patterns differ based on maturity. CYP3A4 is mainly expressed in adult liver, CYP3A5 in extrahepatic tissues, and CYP3A7 in fetal liver [8]. Activation or inhibition of the induction process of such enzyme systems can have severe consequences. Witkamp et al. [9] 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 cytochrome P450 3A4 metabolic intermediate complex in both microsomes and hepatocytes.

The induction of CYP3A4 is mainly regulated by the novel orphan receptor or pregnane X receptor (PXR) [10, 11], but other receptors including the constitutively active receptor (CAR) and, indirectly, the glucocorticoid receptor (GR) are involved [12,13]. Dexamethasone, one of the glucocorticoids, influences several aspects of CYP3A 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 [14]. Information on how or if ET fits...
into the induction processes of rat CYP3A is limited or not available. Therefore, this report was designed to study the effects of administering ET in vivo on the induction of CYP3A family using dexamethasone as a specific CYP3A inducer for similarity and to evaluate the in vitro interaction of ET with CYP3A using ketoconazole as specific inhibitor. Ergotamine and similar ergot alkaloids are extremely susceptible to photolytic and air oxidation, hydration and isomer formation at the C-8 position of the ergolene ring. Therefore, ergotamine isomer was included in this study.

2. 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.

Sprague-Dawley rats (n = 30 rats; BW ~250 g) were allowed ab libitum access to water and chow. Rats were randomly assigned to treatment and treated intraperitoneally for 4 consecutive days with one of the following treatments: 1) Control treatment (n = 5 rats, injections of 0.5 ml of corn oil); 2) DXM treatment (n = 20 rats, injections of 100 mg/kg dexamethasone in 50% of corn oil); and 3) ET treatment (n = 5 rats, injections of 100 mg/kg Ergotamine in 0.5 ml of corn oil). Approximately 24 h after the last injection (except in DXM treatment), each rat was anesthetized with chloroform, decapitated, and liver harvested. Livers were stored at –20 °C until microsomes were prepared. In the DXM treatment, five rats were removed each day for time dependence evaluation and livers were harvested in the same manner.

Michaelis-Menten kinetics was used to evaluate the interaction of CYP3A with ET and the linearity of the Lineweaver-Burk plot of 1/V versus 1/S 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 ketocazole (3.0 and 5.0 μM).

Liver microsomes were prepared as reported by Moubarak and Rosenkrans [15]. Briefly, liver tissue (3 - 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, pH 7.4)) with ice-cold medium using a precooled blender for 10 to 20 sec and further homogenized using a Polter-Elvehjem (5×). The homogenate was sequentially centrifuged at 800 g for 10 min, at 13,500 g for 20 min collecting the supernatant and then at 105,000 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% v/v glycerol. The protein concentration after resuspension was approximately 40 mg/ml.

Aliquots of microsomal suspensions were stored at –20°C and were used within 20 to 30 days. Protein concentration was determined by either the method of Lowry [16] using serum albumin as standard or by BCA Protein Assay (Pierce Chemical kit no. 23225).

Ergotamine (ET) and its isomer (ET_iso) were used as substrates to assay presumptive CYP3A4 activity in vitro. Our standard assay methods for the metabolism of ET were analyzed in 330 μl of assay medium, 100 μl of co-factor generating system (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 MgCl₂. 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 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 (M1, M2 from ET and M1_iso and M2_iso from ET_iso) using the HPLC method described by Moubarak and Rosenkrans [15].

Induction data for the dexamethasone treated rats were analyzed using one-way ANOVA with day as the independent variables and concentration of remaining substrate or metabolite as the dependent variables. Ergotamine and dexamethasone induction data after 4 days of treatment were analyzed using one-way ANOVA with treatment as the independent variable and concentration of remaining substrate or metabolites as the dependent variables. The kinetics data of ketoconazole inhibition was interpreted using the double reciprocal plot method of 1/S vs 1/V.

3. RESULTS AND DISCUSSION

Data in Figure 1 represents 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 dexamethasone, a specific inducer of CYP3A. Dexamethasone induced CYP3A activity in rats to a significant (P < 0.01) level only after the third and the fourth days of treatments. These results indicate that the mechanism of DXM induction of rat liver microsomal CYP3A was time dependent, and it took at least three to four successive days of treatments to reach the maximum level of activity. Liver microsomes from rats pretreated with dexamethasone were five times more (P < 0.01) active than microsomes from
Figure 1. The effects of intraperitoneal injections of dexamethasone (DXM) on the induction of rat liver CYP3A activity over four consecutive days of injection. Comparisons were made within substrate (ET or ET isomer). Bars containing the same letter were not different significantly (P > 0.05).

Figure 2. The amount of substrate (ET or ET isomer) remaining after incubation with rat hepatic CYP3A preparation from control (CON), Dexamethsone (DXM) and Ergotamine (ET) treated rats. Comparisons between treatments were made within substrate. Bars containing the same letter were not different significantly (P > 0.05).

Figure 3. Lineweaver-Burk plots of rat hepatic CYP 3A4 activity in 0, 3, and 5 μM of ketoconazole.
ET was different from that for ketoconazole in rat hepatic CYP3A.

REFERENCES

[1] Porter, T.D. and Coon, M.J. (1991) Cytochrome P-450. Multiplicity of isozymes, substrates, and catalytic and regulatory mechanisms. *Journal of Biological Chemistry*, 266, 13469-13472.

[2] Brosen, K. (1993) The pharmacogenetics of the selective serotonin reuptake inhibitors. *Journal of Clinical Investigation*, 71, 1002-1009.

[3] Pollock, B.G. (1994) Recent developments in drug metabolism of relevance to psychiatrists. *Harvard Review of Psychiatry*, 2, 204-213. doi:10.3109/10675229409017138

[4] DeVane, C.L. (1994) Pharmacokinetics of the newer antidepressants: Clinical relevance. *American Journal of Medicine*, 97, 13S-23S. doi:10.1016/0002-9343(94)90359-X

[5] Wright, M.C. and Paine, A.J. (1994) Induction of the cytochrome P450 3A subfamily in rat liver correlates with the binding of inducers to a microsomal protein. *Biochemical and Biophysical Research Communications*, 201, 973-979. doi:10.1006/bbrc.1994.1797

[6] Ball, S.E., Maurer, G., Zollinger, M., Ladona, M. and Vickers, A.E. (1992) Characterization of the cytochrome P-450 gene family responsible for the N-dealkylation of the ergot alkaloid CQA 206-291 in humans. *Drug Metabolism and Disposition*, 20, 56-63.

[7] Moorchhala, S.M., Lee, E.J., Hu, G.T., Koh, O.S. and Becket, G. (1989) Effects of bromocriptine on hepatic cytochrome P-450 monooxygenase system. *Japanese Journal of Pharmacology*, 49, 285-291. doi:10.1254/jjp.49.285

[8] Thummel, K.E. and Wilkinson, G.R. (1998) In vitro and in vivo drug interactions involving human CYP3A. *Annual Review of Pharmacology and Toxicology*, 38, 389-430. doi:10.1146/annurev.pharmtox.38.1.389

[9] Wittkamp, R.F., Nijmeijer, S.M., Monshouwer, M. and Van Miert, A.S. (1995) The antibiotic tiamulin is a potent inducer and inhibitor of cytochrome P4503A via the formation of a stable metabolic intermediate complex. Studies in primary hepatocyte cultures and liver microsomes of the pig. *Drug Metabolism and Disposition*, 23, 542-547.

[10] Lehmann, J.M., McKee, D.D., Watson, M.A., Willson, T.M., Moore, J.T. and Kliewer, S.A. (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *Journal of Clinical Investigation*, 102, 1016-1102. doi:10.1172/JCI3703

[11] Moore, L.B., Parks, D.J., Jones, S.A., Bledsoe, R.K., Consler, T.G., Stimmel, J.B., Goodwin, B., Liddle, C., Blanchard, S.G., Willson, T.M., Collins, J.L. and Kliewer, S.A. (2000) Orphan nuclear receptors constitutive androstan receptor and pregnane X receptor share xenobiotic and steroid ligands. *Journal of Biological Chemistry*, 275, 15122-15127. doi:10.1074/jbc.M001215200

[12] Sueyoshi, T., Kawamoto, T., Zelko, I., Honkakoski, P. and Negishi, M. (1999) The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *Journal of Biological Chemistry*, 274, 6043-6046. doi:10.1074/jbc.274.10.6043

[13] Pascucci, J.M., Drocourt, L., Fabre, J.M., Maurel, P. and Vilarem, M.J. (2000) Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha expression in human hepatocytes: Synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Molecular Pharmacology*, 58, 361-372. doi:10.1006/mpat.2000.3219

[14] Honkakoski, P. and Negishi, M. (2000) Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochemical Journal*, 347, 321-337. doi:10.1042/0264-6021:3470321

[15] Moubarak, A.S. and Rosenkranzs Jr, C.F. (2000) Hepatic metabolism of ergot alkaloids in beef cattle by cytochrome P450. Biochem. *Biochemical and Biophysical Research Communications*, 274, 746-749. doi:10.1006/bbrc.2000.3210

[16] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.J. (1951) Protein measurements with folin phenol reagent. *Biological Chemistry*, 193, 265-275.

[17] Moubarak, A.S., Rosenkranzs Jr., C.F. and Johnson, Z.B. (2003) Modulation of cytochrome P450 metabolism by ergonovine and dihydroergotamine. *Veterinary & Human Toxicology*, 45, 6-9.

[18] Lampen, A., Christians, U., Guengerich, F.P., Watkins, P.B., Kolars, J.C., Bader, A., Gonschior, A.K., Dralle, H., Hackbarth, I. and Sewing, K.F. (1995) Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metabolism and Disposition*, 23, 1315-1324.

[19] Christians, U., Schmidt, G., Bader, A., Lampen, A., Schottmann, R., Linck, A. and Sewing, K.F. (1996) Identification of drugs inhibiting the in vitro metabolism of tacrolimus by human liver microsomes. *British Journal of Clinical Pharmacology*, 41, 187-190. doi:10.1111/j.1365-2125.1996.tb00181.x

[20] Althaus, M., Retzow, A., Castell, J.V., Gomez-Lechon, M.I., Amalou, Z., Rose, T. and Appel, K. (2000) In vitro identification of the cytochrome P450 isoform responsible for the metabolism of alpha-dihydroergocryptine. *Xenobiotica*, 30, 1033-1045. doi:10.1080/00498250010002261