Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 85 (2008) 80–85
RVS
www.elsevier.com/locate/rvsc

Cytochrome P450 3A, NADPH cytochrome P450 reductase and cytochrome b_5 in the upper airways in horse

E. Tydén *, L. Olsén, J. Tallkvist, H. Tjälve, P. Larsson

Department of Biomedical Sciences and Veterinary Public Health, Division of Pathology, Pharmacology and Toxicology, Swedish University of Agricultural Sciences, Box 7028, Uppsala, S-750 07, Sweden

Accepted 16 September 2007

Abstract

Gene and protein expression as well as catalytic activity of cytochrome P450 (CYP) 3A were studied in the nasal olfactory and respiratory mucosa and the tracheal mucosa of the horse. We also examined the activity of NADPH cytochrome P450 reductase (NADPH P450 reductase), the amount of cytochrome b_5 and the total CYP content in these tissues. Comparative values for the above were obtained using liver as a control. The CYP3A related catalytic activity in the tissues of the upper airways was considerably higher than in the liver. The CYP3A gene and protein expression, on the other hand, was higher in the liver than in the upper airway tissues. Thus, the pattern of CYP3A metabolic activity does not correlate with the CYP3A gene and protein expression. Our results showed that the activity of NADPH P450 reductase and the level of cytochrome b_5 in the relation to the gene and protein expression of CYP3A were higher in the tissues of the upper airways than in the liver. It is concluded that CYP3A related metabolism in horse is not solely dependent on the expression of the enzyme but also on adequate levels of NADPH P450 reductase and cytochrome b_5.

Keywords: Cytochrome P450; CYP3A; Extrahepatic metabolism; Cytochrome b_5; NADPH P450 reductase; Horse

1. Introduction

The cytochrome P450 (CYP) enzymes catalyze the oxidative biotransformation of various endogenous as well as exogenous materials, including drugs and pesticides, food and environmental contaminants (Guengerich, 1999; Raunio et al., 2005; Rodriguez-Antona et al., 2000; Yamazaki et al., 1996). In addition to the liver CYP-enzymes are present in many extrahepatic tissues, such as the mucosa of the intestines, trachea, bronchi and bronchioli, and the olfactory and respiratory nasal mucosa (Ding and Kaminsky, 2003; Gonzalez, 1988).

The CYP-enzymes have mainly been studied in man and laboratory animals, whereas little is known about the CYP-enzymes in domestic animals. In particular there are few reports on the presence and catalytic activity of CYP-enzymes in extrahepatic tissues of domestic animals.

As concerns the horse, we have previously shown CYP3A4 and CYP2A6/2B6 immunoreactivity in some cells of the nasal olfactory, nasal respiratory, tracheal, bronchial and bronchiolar mucosa (Larsson et al., 2003). In vitro metabolic experiments with microsomal preparations also demonstrated that these tissues have a high capacity to form tissue-bound metabolites of the mycotoxin aflatoxin B_1(AFB_1), which is known to be bioactivated by CYP-enzymes, including CYP3A4 and CYP2A6 (Larsson et al., 2003). In another study we have examined mRNA expression, catalytic activity and immunohistochemical localization of CYP3A in the intestinal enterocytes in horse (Tyden et al., 2004). The highest levels of these parameters were observed in the proximal parts of the intestines, with decreasing amounts towards the distal parts of the intestinal tract. CYP3A is an important CYP-form with wide substrate specificity. CYP3A-mediated metabolism in the

* Corresponding author. Tel.: +46 18 673171; fax: +46 18 504144.
E-mail address: Eva.Tyden@bvf.slu.se (E. Tydén).

0034-5288/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2007.09.012

intestinal epithelial cells may reduce the amounts of drugs or chemicals available for absorption and thus limit their positive or negative effects.

In the present study we have examined the gene and protein expression and the catalytic activity of CYP3A in the nasal olfactory and respiratory mucosa and in the tracheal mucosa in horse. At these sites CYP3A may have a local function as a first line of defence against inhaled xenobiotics. CYP3A may also be important in tissue-specific bioactivation of xenobiotics in these tissues. In horses the olfactory and respiratory tissues may be exposed to mycotoxins and other xenobiotics via inhalation of contaminated feed-dust particles (Burg and Shotwell, 1984; Sorenson et al., 1981) and for substances that are bioactivated to toxic metabolites noxious effects may then be induced.

In this study the CYP3A related metabolism in the tissues of the upper airways in horse was examined using the selective fluorescent probe 3-(3,4-difluorobenzyloxy)-5,5-dimethyl-4-(4-methylsulfonylphenyl)-5H-furan-2-one (DFB). CYP3A has been shown to play a major role in the metabolism of DFB (Chauret et al., 1999). Thus, DFB is mainly metabolized by CYP3A4 and in addition to a low extent by CYP3A5. The DFB-metabolizing capacity of other CYP-isoenzymes, such as CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, CYP2C8, CYP2C9 and CYP2C19, was found to be very low or undetectable (Chauret et al., 1999).

It is known that the provision of electrons by NADPH cytochrome P450 reductase (NADPH P450 reductase) and cytochrome b_5 is rate limiting in the catalytic CYP-cycle (Nakajima et al., 2002; Schenkman and Jansson, 2003; Yamazaki et al., 1996). We therefore also examined the activity of NADPH P450 reductase and the amount of cytochrome b_5 in the upper airway tissues in horse. In addition we examined the total CYP content in these tissues. To obtain comparative data experiments were performed with the liver.

2. Materials and methods

2.1. Chemicals and reagents

TRIzol® reagent was obtained from Invitrogen AB, Sweden. Ribogreen and Picogreen were from Molecular Probes Inc., Eugene, OR, USA. QuantiTect™ SYBR® Green RT-PCR Kit was from Qiagen, Inc., Valencia, CA, USA. Primers were custom-synthesized by Cybergene, Huddinge, Sweden. Strataprep™ PCR Purification Kit was from Stratagen, La Jolla, CA, USA. 3-(3,4-difluorobenzyloxy)-5,5-dimethyl-4-(4-methylsulfonylphenyl)-5H-furan-2-one (DFB) and 3-hydroxy-5,5-dimethyl-4-(4-methylsulfonylphenyl)-5H-furan-2-one (DFH) were generous gifts from Merck Frosst Centre for Therapeutic Research, Quebec, Canada. Primary rabbit anti-human CYP3A4-antibody was obtained from Chemicon International, Inc., Temecula, CA, USA. Recombinant human CYP3A4 protein was purchased from BD Biosciences, Inc, San José, CA, USA. SDS–PAGE and nitrocellulose membranes were from, Bio-Rad, CA, USA. ECL western blotting detection reagents and the secondary antibody (donkey anti-rabbit IG) conjugated with horseradish peroxidase were from Amersham Biosciences, Uppsala, Sweden. Cytochrome *c* Reductase (NADPH) Assay Kit was from Sigma–Aldrich, Inc., Saint Louis, Missouri, USA. Complete protease inhibitor cocktail tablets and Titan™ One Tube RT-PCR Kit were from Roche Molecular Chemicals, Basel, Switzerland. Other chemicals were obtained from regular commercial sources.

2.2. Animals and sample collection

Tissues from healthy Swedish standard bred trotters ($n = 12$) were collected at a local slaughterhouse. The horses were 3–9 years old and of both sexes. Samples were collected from nasal olfactory mucosa, nasal respiratory mucosa, tracheal mucosa and liver (Larsson et al., 1989). For preparation of RNA the mucosal surfaces were scraped smoothly with a scalpel, collected in RNase free plastic tubes and snap-frozen at −78 °C in hexane and dry ice, as described previously (Tyden et al., 2004). Liver-tissue RNA was prepared in the same way. For preparation of microsomes the various tissues were collected in 0.9% NaCl (4 °C) before transport to the laboratory.

2.3. RNA preparation and real-time RT-PCR

Total RNA was prepared with TRIzol according to the manufacturer's recommendation. The resulting RNA pellet was suspended in 50 μl diethyl pyrocarbonate treated-water. The RNA-purity and approximate concentration were determined spectrophotometrically by measuring the absorbance at 260 and 280 nm after dilution of the sample 1:500 in H_2O. Only samples with 260/280 nm ratio exceeding 1.8 were selected. Visualization of the 28 S and 18 S ribosomal RNA was used to check the integrity of the RNA. RNA samples were run on an ethidium bromide-stained 1% agarose gel containing 18% formaldehyde at 60 V for 2 h. The RNA samples were stored at −80 °C before use. The CYP3A sequence was quantified by real-time RT-PCR, as described by Tyden et al. (2004), applying following primers: 5′-CAG CCT GGT GCT CCT CTA TC-3′ (forward) and 5′-TCA ACA CCC TTA CGG TAG CC-3′ (reverse). These primers were designed based on conserved regions of the CYP3A4 cDNA sequences from human, pig and rat (Tyden et al., 2004). The length of the generated PCR-product was 164 bp.

The RNA samples were pretreated with DNase prior to the real-time RT-PCR reaction according to the method of (Huang et al., 1996). The exact RNA-concentration was measured with Ribogreen (molecular probes) using a microplate reader (Wallac 1420 VICTOR^2, soft-