Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part D 2 (2007) 91 – 100
CBP
www.elsevier.com/locate/cbpd

The chimpanzee cytochrome P450 3A subfamily: Is our closest related species really that similar?

Eric T. Williams [a], Katherine R. Schouest [b], Małgorzata Leyk [c], Henry W. Strobel [a,*]

[a] Department of Biochemistry and Molecular Biology, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030, USA
[b] Department of Molecular Genetics, M.D. Anderson Cancer Center, University of Texas, Houston, TX 77030, USA
[c] Lilly Research Laboratories, Lilly Corporate Center, Drop Code 6036, Eli Lilly and Company, Indianapolis, IN 46285, USA

Received 4 December 2006; received in revised form 19 December 2006; accepted 24 December 2006
Available online 26 January 2007

Abstract

With the release of the chimpanzee genomic database, much work has been accomplished to understand more fully the closest related species to humans. This study investigates the cytochrome P450 3A (CYP3A) subfamily and examines differences which may be expected between chimpanzees and humans in regards to CYP3A metabolism. A previous publication had reported the presence of five putative chimpanzee CYP3A isoforms, as compared to the four in humans (Williams ET et al., Mol Phylogenet Evol 33, 300–8). Based on the previous report, the chimpanzee CYP3A5 should have had a different C-terminus than its human counterpart; therefore, CYP3A5 and CYP3A67 were cloned. The CYP3A5 clone obtained disputes the previous prediction and confirms that the nucleotide similarity between the two species is 99.7%. While CYP3A67 is most closely related to CYP3A7, with significant differences in the amino acid sequences. Also, the mRNA expression of CYP3A67 can rival the expression of CYP3A4 in the tissues analyzed. CYP3A7 was not found to be expressed in any chimpanzee tissue examined. Total CYP3A protein expression was not significantly different between chimpanzees and humans. Metabolism assays using benzphetamine and erythromycin with chimpanzee liver microsomes did not reveal major differences between chimpanzees and humans. In conclusion, adult CYP3A metabolism may not be significantly different between chimpanzees and humans.
© 2007 Elsevier Inc. All rights reserved.

Keywords: CYP3A4; CYP3A5; CYP3A7; CYP3A43; CYP3A67; Chimpanzee; Human

1. Introduction

Recently, the beginnings of the new chimpanzee (*Pan troglodytes*) genomic database were received with much fanfare since its publication in the September 1, 2005, issue of *Nature*. The genomes of many species have been sequenced, including the fruit fly, human, mouse, rat, and yeast. The human genome is especially important since it lays the groundwork for understanding our own species. However, none of the other genomes sequenced have been of a species as similar to the human, until now. With the release of the chimpanzee genomic database, a sizable influx of publications comparing chimpanzee and human genes and gene families may be expected. This paper explores the cytochrome P450 3A subfamily (CYP3A) in the chimpanzee in terms of its potential relevance for studies of drug metabolism.

While the chimpanzee will most likely never be an animal model for drug discovery, the importance is in the question "Is Our Closest Related Species Really That Similar?" in regards to the CYP3A subfamily. The similarity between the chimpanzee and human CYP3As may indicate if an animal model could be truly reflective for humans. If the chimpanzee CYP3As are not significantly different, then a lower primate could suffice as an animal model. On the other hand, if a significant difference exists between chimpanzee and human CYP3As, then the suggestion is that a predictive animal model may not exist.

The cytochrome P450 superfamily (P450) exists in most eukaryotes. Thousands have been discovered and can be organized into a few hundred families. Each family shares 40% sequence similarity between family members, with subfamilies sharing 55% or greater sequence similarity (Nelson et al., 1996). One subfamily, the cytochrome P450 3A subfamily, is most

* Corresponding author. 6431 Fannin, MSB 6.200, Houston, TX 77030, USA. Tel.: +1 713 500 6078; fax: +1 713 500 0652.
E-mail address: Henry.W.Strobel@uth.tmc.edu (H.W. Strobel).

1744-117X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbd.2006.12.004

known for its metabolism of substrates with diverse structures, and in particular drugs with therapeutic benefit. The best known member of the CYP3As is the human cytochrome P450 3A4 (CYP3A4).

CYP3A4 is an enzyme of major importance to pharmaceutical companies when designing new therapeutic compounds. It is the most abundant P450 (Guengerich, 2005) and metabolizes the largest percentage of clinically-utilized drugs (Evans and Relling, 1999), including erythromycin, cyclosporin, warfarin, and 17β-estradiol. Substrate metabolism by CYP3A4, however, can be a double-edged sword. On one hand, P450 metabolism can be viewed as protective, in the case of xenobiotics (Kivisto et al., 2004). These exogenous compounds are made more hydrophilic for increased excretion. On the other hand, metabolism can be detrimental, as is the case with benzo[a]pyrene (Shimada et al., 1989). Regulation of CYP3A4 is just as varied as the substrates metabolized. Grapefruit juice (6′,7′-dihydroxybergamottin) inhibits its activity through a mechanism-based method (Kakar et al., 2004) and a component of the dietary supplement St. John's Wort (hyperforin) induces its mRNA expression (Komoroski et al., 2004).

Three more CYP3As exist in humans — CYP3A5, CYP3A7, and CYP3A43. The expression of CYP3A5 is polymorphic, but can equal the expression of CYP3A4 (Kuehl et al., 2001). CYP3A7 is known as the predominant fetal isoform (Komori et al., 1990), but is expressed in low amounts in adults (Burk et al., 2002). CYP3A43 was the latest human CYP3A to be discovered; therefore, less is known about it except that its expression is much lower than CYP3A4 (Williams et al., 2004a).

The chimpanzee is considered to be the species most closely related to humans, with a 1.23% divergence between their genomic sequences (Chimpanzee Sequencing and Analysis Consortium, 2005). That small percentage makes a huge phenotypic difference. One reported genomic difference is the fusion of the chimpanzee chromosomes 2a and 2b (formerly known as 12 and 13) to give rise to what appears as the human chromosome 2. Two additional genotypic differences with the chimpanzee, as compared to humans, are an extra alpha-hemoglobin gene and fewer *Alu* repeats. A phenotypic difference between the species is the chimpanzee's insensitivity to hearing in the range of 2 to 4 kHz, which is where most human speech is audible. Despite these genotypic and phenotypic differences, most molecular pathways appear to be rather similar between chimpanzees and humans, including the expression and metabolic profiles of the CYP3As.

The first publication to look at chimpanzee CYP3As suggested five isoforms versus four found in humans (Williams et al., 2004b). The four human isoforms — CYP3A4, CYP3A5, CYP3A7, and CYP3A43 — are also found in the chimpanzee plus CYP3A67, which is currently only known to exist in chimpanzees. While that publication only predicted the sequences of the five isoforms based on the chimpanzee genomic database, the cloning of CYP3A5 and CYP3A67 will demonstrate that the previous predictions were inaccurate in some ways. In addition this study goes further than the previous study by presenting evidence for similar mRNA and protein expression levels for CYP3A4, CYP3A5, and CYP3A43, but showing a difference in the chimpanzee with CYP3A7 caused by CYP3A67. Also, the substrate metabolism may be similar between the two species. The work presented here will begin to answer the question "Is Our Closest Related Species Really That Similar?" by laying the foundation for the characterization of the chimpanzee CYP3A subfamily.

2. Materials and methods

2.1. Chimpanzee tissues

The chimpanzee tissues were obtained from the Yerkes Primate Center at Emory University in Atlanta, Georgia. The tissues included four samples from each of the following tissues: heart, kidney, liver and spleen. If possible, a sample from each tissue was obtained from the same animal, but this was not possible in all circumstances. Supplemental Table 1 presents the background information of each animal that samples were obtained from. Fetal chimpanzee tissue and adult intestinal tissue could not be obtained.

2.2. RNA isolation

RNA was isolated from each sample as previously described (Williams et al., 2004a); however, TRIZol (15596018; Invitrogen; Carlsbad, CA) was used in the place of RNA STAT-60. Two concentrations of RNA were prepared for use — 1 μg/μL for gene cloning and 25 ng/μL for Quantitative Real-Time PCR.

2.3. Gene cloning

The isolated RNA at a concentration of 1 μg/μL was used to create an RNA pool composed of equal amounts of the four chimpanzee liver samples. The RNA pool was used with the protocol outlined for the BD SMART PCR cDNA Synthesis Kit (634902; BD Biosciences Clontech; Mountain View, CA), which was used for first-strand synthesis and PCR amplification to create the cDNA pool used for gene cloning. All primers were synthesized by SeqWright (Houston, TX).

For the cloning of the CYP3A5 coding region the forward primer was ATGGACCTCATCCCAAATTTGGCG and the reverse primer was TTATTGACTAAGTTGAAATCTCTGGTGTTCTGG. The forward primer for CYP3A67 was ATGGACCTCATCCCAAATTTGGCA and the reverse primer was TCAGGCTCCACTCACAGTCTCATCCC. The primers are designed to capture the entire predicted coding region for their respective isoform.

Based upon the coding region sequence obtained for each isoform, a gene-specific primer was designed for each isoform to be used with a generic primer to capture the 5′-and 3′-UTRs (untranslated region). The gene-specific primers are located far enough within the coding region to allow sufficient sequence overlap to verify that the sequences are specific for their respective isoform. For the amplification of the 5′-UTRs, the generic primer used was

Download English Version:

https://daneshyari.com/en/article/1978891

Download Persian Version:

https://daneshyari.com/article/1978891

Daneshyari.com