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Expression profiles of a drug metabolizing enzyme CYP3A62 mRNA in the intestine of rats

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ABSTRACT

The purpose of this study was to examine the expression profiles of a drug metabolizing enzyme cytochrome P450 (CYP) 3A62 mRNA in the intestine and liver of normal male Wistar rats, and its regional differences in the intestinal tract. Nine-week-old male rats were used, and their intestine were divided equally into nine segments. The expression patterns of CYP3A62 mRNA in the intestine and liver were examined using a reverse transcription-polymerase chain reaction (RT-PCR). The PCR products for CYP3A62 mRNA were readily detectable in the intestine of normal male rats, whereas no bands for CYP3A62 mRNA were detected in the liver using similar PCR conditions as those used in the intestine. The expression of CYP3A62 mRNA was found to be high in the jejunum, and stable until the bottom of the small intestine. Only the decreased expression pattern in the bottom of the intestine was contradictory to our previous findings on CYP3A9 and CYP3A18 mRNAs. Unlike other CYPs of the CYP3A subfamily, CYP3A62 was found to be predominant in the intestine rather than the liver of normal male Wistar rats. This suggests that CYP3A62 also has an important role in the detoxification of xenobiotics similar to endogenous substances as an absorptive barrier in the intestine. These findings may help the evaluation of gastrointestinal drug absorption and drug-drug interaction in rats.

Keywords: cytochrome P450, CYP3A62, intestine, Wistar rat, RT-PCR, expression profile

INTRODUCTION

The cytochrome P450-dependent microsomal monooxygenase system consists of a flavoprotein, cytochrome P450 reductase, and a multigene superfamily of hemeproteins (the cytochrome P450s - CYPs). CYPs belonging to the CYP3A subfamily are among the most abundant, being predominantly expressed in the liver, but are also present in the gut, leukocytes, and brain (Ding and Kaminsky 2003; Miksys and Tyndale 2002). In addition, the CYP3A subfamily may contribute to the metabolism of approximately 50% of marketed drugs (Wringhton and Ring 1999). Thus, the CYP3A subfamily plays an important role in the detoxification of xenobiotics as well as endogenous substances.

In rats, five CYP3A cDNAs have been characterized; CYP3A1 (Gonzalez et al., 1985), CYP3A2 (Gonzalez et al., 1986), CYP3A9 (Wang et al., 1996), CYP3A18 (Nagata et al., 1996; Strotkamp et al., 1995), and CYP3A23 (Kirita and Matsubara 1993; Komori and Oda 1994). CYP3A23 was, however, identified as having the same structure as CYP3A1 (Nagata et al., 1999). The existence of these in the intestine of rats has been demonstrated by various experimental techniques, whereas some have reported that the CYP3A subfamily is not expressed in this organ (Chiba et al., 1997; de Waziers et al., 1990; Kolars et al., 1992; Watkins et al., 1987; Zhang et al., 1996). Therefore, we have examined the expression profiles of the CYP3A subfamily in the intestine of normal male Wistar rats, and previously clarified that CYP3A1 and CYP3A2 mRNAs are not detected in the intestine of rats, but CYP3A9 and CYP3A18 mRNAs are readily detectable using a reverse transcription-polymerase chain reaction (RT-PCR) (Takara et al., 2003). However, Matsubara et al (2004) demonstrated the expression of a novel CYP3A form, CYP3A62, in the intestine of Sprague-Dawley rats.

In the present study, the expression profiles of CYP3A62 mRNA were examined in both the intestine and liver of normal male Wistar rats using RT-PCR. In addition, the present findings on CYP3A62 were compared with those of other CYP3A isoforms (Takara et al., 2003).

MATERIALS AND METHODS

Animals

Nine-week-old male Wistar rats (240 to 250 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). After an overnight fast with free access to water, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (Nembutal injection®, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and sacrificed by exsanguination *via* the aorta in the abdomen. Animal experimental protocols were carried out in accordance with the Guidelines for Animal Experimentation at Kyoto Pharmaceutical University.

Removal of Tissue and RNA Extraction

The removal and the extraction of total RNA from rat tissue samples were carried out as previously reported (Takara et al., 2003). That is, the intestine was excised quickly after exsanguination, and rinsed in ice-cold 0.9% NaCl solution. The intestine (total of ca. 90 cm) was divided into three parts; upper (almost corresponding to the duodenum, ~30 cm immediately distal to the pyloric valve), middle (almost corresponding to the jejunum, the 30 cm between the upper and lower sites), and lower (almost corresponding to the ileum, the last 30 cm before the cecum). Each part was further divided into three sections (about 10 cm each), and the centers (1 cm distal) were cut out to obtain nine segments. These were designated as S1, S2, S3, S4, S5, S6, S7, S8, and S9, in the direction of the duodenum to the ileum. The liver was excised quickly after exsanguination, and divided into parts (3 mm squares). Tissue samples of both the intestine and liver were collected from three rats, immersed quickly into RNA*later*™ (Sigma-Aldrich Chemical Co., St. Louis, MO), and stored at –80°C until RNA extraction.

For RNA extraction, 3-mm squares of tissue samples were homogenized in RNA*later*™ using a pestle, then total RNA was extracted using a GenElute™ Mammalian Total RNA kit (Sigma-Aldrich) as previously reported (Takara et al., 2003).

Table 1: Gene-specific oligonucleotide primers used in PCR reaction for mRNA detection

Gene		Sequence	Fragment size[a)]
CYP3A62	Forward Primer	5'-GGA AAT TCG ATG TGG AGT GC-3'	544
	Reverse Primer	5'-AGG TTT GCC TTT CTC TTG CC-3'	
GAPDH	Forward Primer	5'-CGA TCC CGC TAA CAT CAA AT-3'	391
	Reverse Primer	5'-GGA TGC AGG GAT GAT GTT CT-3'	

[a)]Area amplified refers to sequences deposited at GenBank with accession numbers as follows: AB084894 for CYP3A62 (937–1481) and AF106860 for GAPDH (1083–1473). The primer sequences for GAPDH were designed by Primer3 software (Rozen and Skaletsky 2000).

RT-PCR

RT-PCR analysis was performed as described previously (Takara et al., 2003). Aliquots (0.3 µg) of total RNA were used for reverse transcription with an RNA PCR kit (AMV) ver. 2.1 (TakaraBio Inc., Shiga, Japan). The RT reaction was conducted in 15 µl RT reaction mixture, and this was incubated at 30°C for 10 min and subsequently at 42°C for 30 min in a thermal cycler (iCycler Thermal Cycler, Bio-Rad Laboratories, Inc., CA). The RT reaction was terminated by heating to 99°C for 5 min, followed by cooling at 5°C for 5 min in a thermal cycler (Bio-Rad).

PCR primer sequences for amplification of CYP3A62 and glyceraldehyde-3-phos -phate dehydrogenase (GAPDH) are listed in Table 1 (Matsubara et al., 2004), and were synthesized by Proligo Japan K.K. (Kyoto, Japan). PCR amplification of cDNA was performed in a total reaction volume of 25 µl including 5 µl of RT product, using an RNA PCR kit (AMV) ver. 2.1 (TakaraBio). After initial denaturation at 95°C for 1 min, both CYP3A62 and GAPDH cDNAs were amplified for 22 cycles with 30 sec at 94°C for denaturation, 1 min at 60°C for annealing and 1 min at 72°C for extension, followed by a final extension period of 10 min at 72°C, using a thermal cycler (Bio-Rad). PCR products were separated on Tris-acetate-EDTA 3% agarose gels containing 100 ng/ml ethidium bromide, and photographed under ultraviolet illumination at 312 nm with a Polaroid camera. Band densities were measured using the computer program NIH Image version 1.63 (National Institutes of Health, Bethesda, MD), and band density ratio (CYP3A62/GAPDH) was calculated.

Statistical Analysis

Results are expressed as percentages of the ratio of CYP3A62 to GAPDH mRNA levels in the S1 region. These data were compared with the S1 region by non-repeated measures one-way analysis of variance followed by the Bonferroni test. The *p* values of less than 0.05 (two-tailed) were considered significant.

RESULTS AND DISCUSSION

In this study, the intestinal expression profile of a drug metabolizing enzyme CYP3A62, isolated and characterized by Matsubara et al (2004), was examined in normal male Wistar rats using RT-PCR. In addition, the findings on CYP3A62 were compared with those of other documented CYP3A isoforms (Takara et al., 2003).

First, the expression of CYP3A62 mRNA was compared in the intestine and liver of

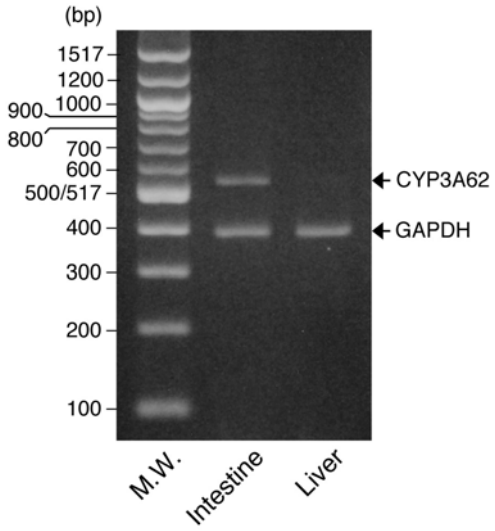

Figure 1: Expression profiles for CYP3A62 mRNA in the intestinal tract and liver of male Wistar rats as determined by RT-PCR. Rats were sacrificed, and their tissues were quickly collected into RNA*later*™. Total RNA was extracted from the tissue using a GenElute™ Mammalian Total RNA kit. PCR products were separated on a 3% agarose gel containing 100 ng/ml ethidium bromide, to visualize the DNA bands. M.W., Molecular weight marker (100 bp DNA ladder).

normal male rats. The 544 bp PCR product for CYP3A62 mRNA was readily detectable in the intestine of normal male rats, whereas there was no band for CYP3A62 mRNA in the liver using the same PCR cycling conditions (Fig. 1). However, a faint band for CYP3A62 mRNA was detected in the normal liver with an increase of the PCR cycle number (data not shown). These results indicate that the expression of CYP3A62 mRNA is predominant in the intestine rather than the liver. This was supported by results from normal male Sprague-Dawley rats, in which the expression level of CYP3A62 mRNA in the intestine was a few-fold higher than in the liver (Matsubara et al., 2004)

Regional differences in CYP3A62 mRNA intestinal expression were also examined. The expression of CYP3A62 mRNA increased from the upper to the lower intestine in normal rats, and was significantly higher in the S4 and S8 than in the S1 region (Fig. 2). In addition, the expression level of CYP3A62 mRNA was similar from S4 to S9 regions (Fig. 2). Matsubara et al (2004) demonstrated that the expression level of CYP3A62 mRNA decreased slightly from the duodenum to the ileum. The discrepancy between our findings and theirs may be explained by differences in rat strain or the removal sites in the intestine. CYP3A62 mRNA was found to be expressed in both the intestine and liver, but predominantly in the intestine of normal male Wistar rats. Also, a regional difference in the intestine was noted in the expression of CYP3A62 mRNA.

Previously, we found that CYP3A9 and CYP3A18 mRNAs are readily detectable in the intestine, whereas CYP3A1 and CYP3A2 mRNAs were undetected by RT-PCR (Takara et al., 2003). In addition, both CYP3A9 and CYP3A18 mRNA levels were high in the duodenum, and decreased from the top to the bottom of

the gut, indicating the regional differences in both CYP3A9 and CYP3A18 mRNA expression. Taking previous reports into consideration, the expression profile of CYP3A62 mRNA in the intestine appears similar to those of CYP3A9 and CYP3A18 mRNAs, but not CYP3A1 and CYP3A2 mRNAs. However, the expression pattern of CYP3A62 mRNA specifically in the bottom of the intestine was different from those of CYP3A9 and CYP3A18 mRNAs (Takara et al., 2003). In contrast to these findings, it is generally accepted that the expression level of CYPs decreases from the top to the bottom of the gut (Kaminsky and Fasco 1991). Consequently, the expression profiles of CYP3A62 mRNA may differ from those of others in the CYP3A subfamily in the intestine of normal male Wistar rats.

In conclusion, CYP3A62 in normal male Wistar rats was found to be predominant in the intestine rather than in the liver, suggesting that it may have an important role in the detoxification of xenobiotics similar to endogenous substances as an absorptive barrier in the intestine.

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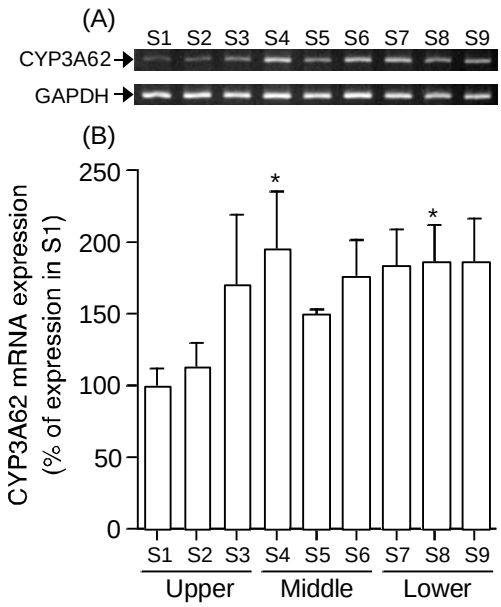

Figure 2: Regional differences in CYP3A62 mRNA expression in the intestinal tract of normal male Wistar rats as determined by RT-PCR. Rats were sacrificed, and their tissues quickly collected into RNA*later*™. Total RNA was extracted from the tissue using a GenElute™ Mammalian Total RNA kit. PCR products were separated on a 3% agarose gel containing 100 ng/ml ethidium bromide, to visualize the DNA bands. (A) Representative electrophoretogram of CYP3A62 and GAPDH. CYP3A62 and GAPDH were 544 bp and 391 bp long, respectively. (B) Data were obtained after densitometric analysis of electrophoretograms derived from three rats. Results are expressed as percentages of the ratio of CYP3A62 to GAPDH mRNA levels in the S1 region. Each bar represents the mean ± S.E. of three rats. * $p<0.05$ significantly different from S1.

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