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Expression of CYP3A4, CYP3A5 and CYP3A7 in human duodenal tissue

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The essential role of cytochrome P450 3A4 (CYP3A4) in human small intestine is well established, and CYP3A5 seems also to be present in most subjects. However, the role of CYP3A7 in the small intestine remains poorly characterized. We have therefore studied the expression of these CYP3A enzymes in the duodenal tissue from 19 patients, using a specific RT-PCR (reverse transcriptase-polymerase chain reaction) method. CYP3A4 and CYP3A5 were present at the mRNA level in the duodenum of 18 and 19 of the 19 patients studied, respectively. In contrast, mRNA for CYP3A7 was not found in the duodenum in any of the patients. These findings strongly suggest that, unlike CYP3A4 and CYP3A5, CYP3A7 is not expressed in human duodenum.

Keywords cytochrome P450 CYP3A human duodenum gastrointestinal

Introduction

Although cytochrome P450 (CYP) enzymes are predominantly expressed in the liver, they are also found in extrahepatic organs such as the gut and lung [1]. The low and variable oral bioavailability of some drugs such as cyclosporin, verapamil and other CYP3A substrates is probably partly due to their first-pass metabolism in the gut wall [2–5]. Furthermore, it has been recently recognized that gut wall is an important site for drug interactions. Enzyme inducers (e.g. rifampicin) and inhibitors (e.g. erythromycin, ketoconazole, itraconazole and grapefruit juice) can alter the gastrointestinal first-pass metabolism of, for example, cyclosporin, verapamil and midazolam, leading to markedly reduced or increased bioavailability [4–9].

CYP3A4, the most abundant CYP3A enzyme in human liver, plays an important role in the oxidative metabolism of a large number of essential drugs [10]. In contrast to CYP3A4, CYP3A5 seems to be present in only 20–25% of adult livers [11, 12]. CYP3A7 was previously thought to be confined to fetal liver, but in two recent studies CYP3A7 mRNA was found in the majority of adult livers studied [13, 14]. However, it is unclear whether CYP3A7 protein is expressed in adult liver.

The presence of CYP3A4 in human small intestine is now well established [15–18], and CYP3A5 protein was also recently found in the small intestine in most of the patients studied [18]. Preliminary studies in a small number of subjects suggest that CYP3A7 may not be expressed in the gut wall [19, 20], but the available data are too scanty to allow evaluation of the role of CYP3A7 in the small intestine.

The primary objective of this study was to gain more information about the expression of CYP3A7 in human small intestine, as compared with CYP3A4 and CYP3A5. To achieve this, we have studied the expression of CYP3A4, CYP3A5 and CYP3A7 in the duodenum of 19 patients, using a highly sensitive and specific reverse transcriptase-polymerase chain reaction (RT-PCR) method.

Methods

Patients

Normal duodenal tissue was obtained from 19 patients (14 males and five females, age range 18–74 years) undergoing routine endoscopy for different gastrointestinal diseases. The tissue used was left-over material from the biopsies taken for routine histological examination. A written informed consent concerning the use of waste tissue for investigative purposes was obtained from each patient. The study protocol was approved by the local

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Ethics Committee. None of the patients was receiving drugs known to induce CYP3A.

RT-PCR

Total RNA was isolated from homogenized duodenal tissue with the method of Chomczynski & Sacchi [21]. cDNA was synthesized from 100 ng of total RNA, using oligo(dT) priming and AMV reverse transcriptase. The RT-PCR method used for specific detection of CYP3A4, CYP3A5 and CYP3A7 has been described in detail elsewhere [22]. In brief, each PCR reaction contained 2.5 μl of the cDNA sample, 1.25 u Taq DNA polymerase, 1.25 u Taq Extender, PCR buffer, Mg^{2+} (final concentration 2.0 mM), dNTP reaction mixture (final concentration 200 μM), 10 pmol of each primer and water to a final volume of 50 μl. Forty PCR cycles were performed: 1.5 min at 94° C, 0.5 min at 53° C (CYP3A5 and CYP3A7) or 55° C (CYP3A4) and 3 min at 72° C.

The primers were designed for specific amplification of CYP3A4, CYP3A5 and CYP3A7, respectively [22]. The functional specificity of the primers was confirmed using full-length CYP3A4, CYP3A5 and CYP3A7 cDNAs as templates. The PCR products obtained with these primers contain at least one intron if cellular DNA instead of cDNA is used in the PCR, allowing detection of possible contamination of cDNA preparations with genomic DNA.

Each PCR reaction included as a negative control a 50 μl aliquot of the reaction mixture without cDNA. cDNA prepared from a liver known to express all three CYP3A enzymes was used as a positive control to confirm the correct functioning of each PCR assay. After PCR, 10 μl of the reaction mixture was electrophoresed in an agarose gel and stained with ethidium bromide.

Results

subjects, while CYP3A7 is absent. Several studies have confirmed the presence of CYP3A4 in human small intestine [15–18]. For example, Lown *et al.* [18] detected CYP3A4 protein and CYP3A4 mRNA in small bowel enterocytes in all the 20 patients studied. In contrast, data on the expression of CYP3A5 in the gastrointestinal tract are scanty. In a recent study, CYP3A5 protein was detected in biopsies taken from small intestine in 14 of the 20 patients studied (70%) [18]. We found mRNA for CYP3A5 in every patient, but mRNA may not be translated into protein in the duodenum of every subject.

With regard to expression of CYP3A7 in human small intestine, the two previous studies do not allow any conclusions to be made [19, 20]. In the study of Kolars *et al.* [19], mRNA from human small bowel enterocytes (obtained apparently from a single subject) was hybridized on Northern blots with oligonucleotides complementary to CYP3A4, CYP3A5 or CYP3A7 cDNAs. Hybridization was detected only with the CYP3A4-specific oligonucleotide. Furthermore, no mRNA for CYP3A5 or CYP3A7 was found after treating five volunteers with rifampicin, an inducer of CYP3A4 [19]. However, Northern blotting is not as sensitive for detection of mRNA as RT-PCR. The same group recently studied the expression of CYP3A enzymes in different regions of the gastrointestinal tract, using tissue obtained from one human organ donor [20]. Both CYP3A4 and CYP3A5 mRNA were found in all regions of the digestive tract, but CYP3A7 mRNA was not detected even with RT-PCR (with the possible exception of small intestine) [20]. Our findings in a relatively large number of subjects confirm these earlier findings and strongly suggest that a significant role for CYP3A7 in the human small intestine can be excluded.

The total amount of CYP3A4 in the small intestine is probably much lower than in the liver, but the small intestine may, however, have the highest content of CYP3A4 after liver [17]. Several recent studies suggest that, compared with CYP3A4, CYP3A5 may be a relatively minor enzyme in the human small bowel [18, 20, 23]. Although