The Clinical Significance of CYP450 in Gastrointestinal Tract

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ABSTRACT

Cytochromes P450 (CYP450) is a super-family of multigenes bound to heme and also a catalysator enzyme. Cytochrome P450s (1, 2, and 3) are the most important enzymes for biotransformation of drugs administered through gastrointestinal tract. The gastrointestinal tract is the first part of immune system against all of oral xenobiotics. Drug interaction may be predicted but it is hardly prevented. Thus, it frequently becomes clinical problem.

CYP450 polymorphism may influence effective drug metabolism, which consequently will affect drug response and good therapeutic effect. Poor metabolizers need only a small dose of drug to bring on drug response but extensive or ultra-rapid metabolizers will need a large dose of drug. The unexpressed CYP2E1, one of the CYP families, may influence cancer incidence. However, it is still controversial.

Keywords: *CYP450, xenobiotics, biotransformation of drugs, gastrointestinal tract*

INTRODUCTION

Drug interaction is a very important clinical problem as well as recovery of diseases and a specific type of adverse effect, which is usually, may be predicted but cannot be prevented. Drug interaction is determined by biotransformation reactions through cytochrome P450s (CYP450s). There are 18 families and 43 sub-families of CYP450s that have been identified by human. Almost all of drug used for drug therapy and administered through gastrointestinal pathway will be metabolized in gastrointestinal wall by phase I biotransformation (phase I metabolism). Drugs endured biotransformation process through CYP450 aid will be activated from previous prodrug form into an active form or will be metabolized into non-active form.[1,2]

Among the CYP450s families, the CYP1, CYP2 and CYP3 are the most important parts in xenobiotics metabolism. They are highly expressed in the liver as well as extra-hepatic tissues including gastrointestinal tract, especially the small intestines.[3] Drugs metabolized in the intestinal wall include cyclosporine, midazolam, NSAIDs, oral anticoagulant, H_2-blocker, proton pump inhibitor.[3,4]

This literature review is focused on the expression and distribution of CYP450 polymorphism (genetic variant) in gastrointestinal tract associated with its clinical significance.

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THE EFFECT OF CYP450 ENZYMES ON BIOTRANSFORMATION OF DRUG

CYP450 is a very strong microsomal absorbant band of 450 nm wavelength, which is resulted through dithionite treatment and additional CO gas (process of microsomal deletion). In addition, P indicates a pigment.

The strong binding of CO and ferrous heme will provide different absorbance spectrum for dithionite and CO. Such spectrum has been found since 1958.[2]

CYP450 is a main catalysator enzyme, which is important in drug biotransformation reaction. The genes of CYP450 family accommodate metabolism of chemical substance, toxic food and drugs.[1] CYP450s are proteins bound to heme (membrane-bound heme-containing proteins), which respond to the metabolism of several endogenous components such as steroid, fatty acids, as well as other drugs and xenobiotics.[3] CYP450s have important role in phase I biotransformation, which occurs in endoplasmic

reticulum as well as in the conjugated cytosolic fraction.[5] Figure 1 shows the schematic mechanisms of oxygen activation and drugs oxidation through multiple steps which indicate that xenobiotics substrate react with the oxidized Fe^{3+} of CYP450 to form an enzyme.

CYP450 accepts electrons of NADPH, which reduce the oxidation complex of CYP450 and xenobiotics (Fe^{2+}). The last step includes an oxygen atom (H_2O) released and the other oxygen atom is transferred into substrate (figure 1). The most dominant CYP450s enzymes in biotransformation of drugs are CYP1, CYP2 and CYP3 (the proportion is

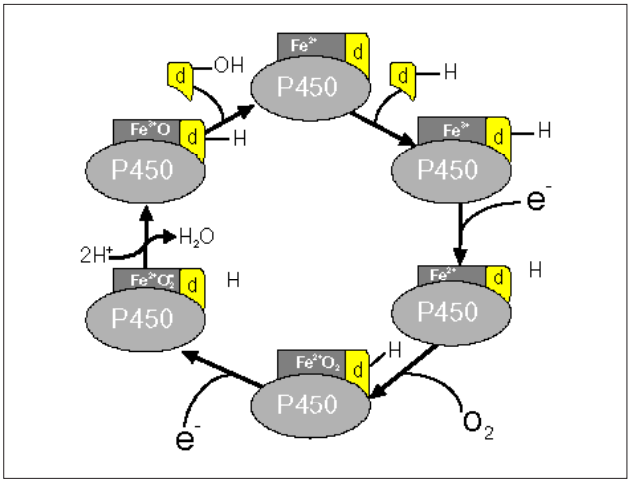

Figure 1. Mechanisms of CYP450s in oxygen activation and drug oxidation.[1]

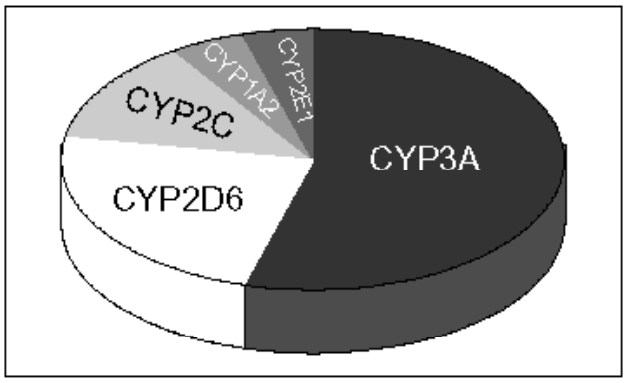

Figure 2. Proportion of drug metabolisms by the most dominant CYP450.[1]

depicted in figure 2).[1]

Increased CYP450 synthesis is correlated to the exposures of drugs and pollutants, which are followed further by increased biotransformation rate and usually also correlated to increased toxicity. In addition, inhibited drug biotransformation will cause increased drug concentration, prolonged pharmacologic response and increased risk of drug-induced toxicity. In fact, the most important factor in biotransformation of drug is genetic polymorphism of CYP450 enzyme.[1] Mutation of CYP450 gene or enzyme deficiency is obviously associated with some disease and induces some CYP450s, which have pro-carcinogenic risk and it also has important role in providing accurate therapy.[4,5,6]

Almost one per third of CYP450s genes is CYP2 and most of its proteins are able to perform hydroxylation reaction against steroid and the other may provide protection against toxins that enter into the body together with food intake.[2] The CYP450 chromosome can be well-detected, either by non-invasive or invasive method. The non-invasive markers may be detected through urine and blood specimens as well as the air of breath; while invasive method needs specimen biopsies.[2,3,7,8]

DISTRIBUTION OF CYP450S IN THE MUCOSA OF GASTROINTESTINAL TRACT

Food, drugs or other xenobiotics entered into gastrointestinal tract will experience biotransformation process (phase I metabolism) on the intestinal wall and almost all of xenobiotic components may activate enzymes into toxic or carcinogenic form.[7] The presence of drugs in gastrointestinal tract is corresponding to its presence in blood. This fact is obvious in the liver donor who had received oral cyclosporine therapy and revealed a detected cyclosporine blood level in the portal vein, when the drug had reached intestines. Similar fact is also found for midazolam.[3]

The evidence should be proven by thorough examination of gastrointestinal mucosa, i.e. specimen biopsies. Gastric, duodenal and colon biopsies can be easily performed by routine endoscopic examination. Specimen biopsies are put in the liquid nitrogen and kept in temperature of -70°C. Subsequently, it is prepared for mRNA examination and followed by CDNA synthesis as well as real-time PCR. The expression and polymorphism of CYP450 enzymes of each specimen biopsy can be analyzed.[3,7,8]

Thorn et al, provided evidence about mRNA expressions of CYP2E1, CYP3A4, and CYP2A5 present in all of gastrointestinal mucosa (stomach, duodenum, right and left colon, rectum). The most frequent mRNA expression of CYP2E1 present in stomach and duodenum, while mRNA of CYP3A4 present in duodenum and mRNA of CYP3A5 present in duodenum and stomach.[3] Von Richter et al, compared p-glycoprotein mRNA expression of CYP3A4 in enterocytes of duodenum or proximal jejunum as well as in the hepatocytes.

It is evident that mRNA expression of CYP3A4 in the enterocytes is 3 times higher and p-glycoprotein expression is 7 times higher compared to

the expressions in the hepatocytes.[8] This study indicated that the expressions of CYP2E1, CYP3A4, CYP2A5 and p-glycoprotein worked together in a synergy as bioavailability barrier or oral drugs. P-glycoprotein is a protein transporter bound to mucosa membrane of gastrointestinal tract, liver and kidney and it is coded by multi-drug resistance genes. Increased expressions of CYP enzymes are associated with increased incidence rate of multi-drugs resistance, which are proven by increased p-glycoprotein expression. In fact, the p-glycoprotein (p-gp) expression increases in all along the gastrointestinal tract, from stomach to the descending colon and slightly reduced at the rectum.[3]

Bergheim et al indicated the distribution of CYP2C, CYP2E1, CYP3A4, and CYP3A5 in colon mucosa. CYP2C expression was more frequent in the ascending colon compared to the descending colon and sigmoid. CYP2E1 expression was not detected in the ascending colon. CYP3A4 expression was similar in all colon and CYP3A5 was more frequent in the descending colon (2 times greater) and the sigmoid (three times greater) compared to the ascending colon.[7]

Figures below are illustrations of mRNAs of CYPs in gastrointestinal mucosa of specimen biopsies analyzed by electrophoresis and immunoblotting (figure 3 and 4) and illustration of mRNA relative

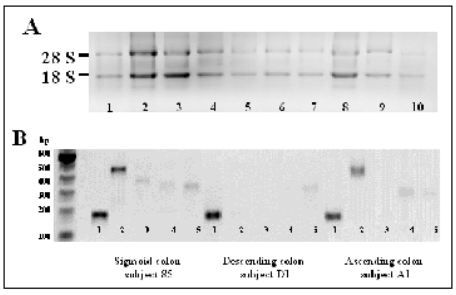

Figure 3. (A) Integrity of colon mucosa biopsy with gel agarose mRNA (lane 1 and 10 positive controls); (B) Photomicrograph image of RT-PCR (lane 1 = Histone 3.3; lane 2 = CYP3A5; lane 3 = CYP2E1; lane 4 = CYP3A4; lane 5 = CYP2C; bp= base pairs)[7]

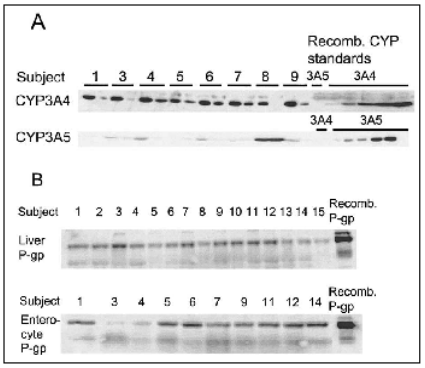

Figure 4. (A) Western blots images of CYP3A4 and CYP3A5 (right lane – hepatocytes, left lane – enterocytes). (B) Western blots of p-glycoprotein from hepatocytes and enterocytes biopsies. Protein load of 5 ug for CYP3A4; 50 ġg for CYP3A5; 40 ġg for hepatocytes P-gp and 20 ġg for enterocytes P-gp.[8]

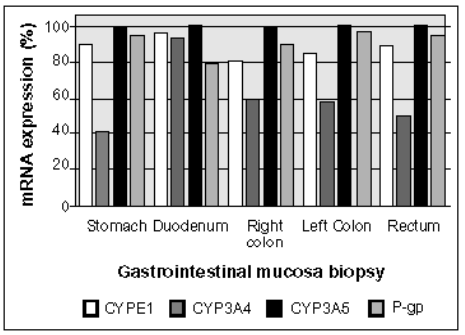

Figure 5. The percentage of gastrointestinal mucosa biopsies with mRNA expressions over detection limit (c.o. > 38).[5]

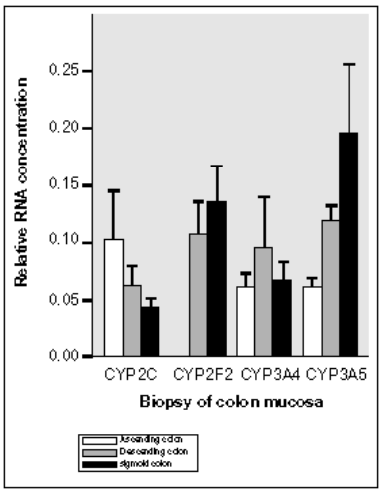

Figure 6. Quantitative analyses on mRNA expressions of CYP2C, CYP2E1, CYP3A4, and CYP3A5 in biopsies of colon mucosa ($p < 0.05$ compared to ascending colon).[7]

concentration (figure 5 and 6).

CORRELATION BETWEEN CYP450 POLYMORPHISM AND GASTROINTESTINAL DISEASE

The CYP2 family is the most abundant type found in human being, which is 1/3 part of CYP450s enzymes. It has multiple polymorphism capacity that may reduce or increase enzyme activity, which clinically can be categorized as poor metabolizers, extensive metabolizers and ultra-rapid metabolizers.[5] CYP isoenzyme is controlled by genetic factors. It has different expression among individuals and is affected by multiple factors such as tobacco smoking, ethanol consumption, environment, disease and genetic factors.[5,9]

Kawamura et al, reported the effect of proton pump inhibitor (PPI), lansoprazole, in inhibiting gastric acid secretion, which is affected by CYP2C19 polymorphism. In homozygous extensive metabolizers (no mutation occur in exon 4 and 5), the effect of gastric acid suppression by PPI is not effective because there is rapid metabolism caused by high enzyme activity of CYP2C19. On the contrary, in poor metabolizers (mutation in both CYP2C19 alleles) effectively inhibit the increased gastric acid secre-

tion.

In heterozygous extensive metabolizers (mutation in an allele), the efficacy of PPI is intermediate. The expression of CYP2C19 polymorphism affects PPI therapeutic responses such as erosive reflux esophagitis therapy and maintenance therapy of reflux esophagitis. There is higher recurrence rate of erosive reflux esophagitis in extensive metabolizers (EM) compared to the poor metabolizers (PM). Consequently, small dose of long-term lansoprazole therapy is not efficient for erosive remission in homozygous and heterozygous extensive metabolizers genotypes. In contrast, poor-metabolizers genotype needs small dose of lansoprazole to maintain erosive remission.[10]

Sapone et al, reported the effect of CYP2C19 (*2 and *3) and CYP3A4 (*1B, *2, and *3), either homozygous or heterozygous extensive metabolizers (homoEM or heteroEM), in eradicating *H. pylori*. It is predicted that the failure of *H. pylori* eradication tends to be higher in the homoEM than the heteroEM. In CYP3A4*1B, the eradication has not been reached. In addition, a positive synergy of CYP3A4 and CYP2C19 heteroEM has affected the eradication of *H. pylori*.[11] High CYP3A4 expression in enterocytes (intestines) may be induced by single oral dose of omeprazole theapy.[8] Mutation of CYP2C19 genotype and its effect on PPI metabolism may influence the *H. pylori* eradication therapy using two or three antibiotics (dual or triple therapy).[12] The high CYP2C19 expression is associated with peptic ulcer.[2] In addition, gastritis complication, peptic ulcer and NSAIDs-induced gastrointestinal bleeding are increasing in CYP2C9 polymorphism.[4]

The high level of CYP450 is correlated to increased risk of cancer incidence, good therapeutic response and drug resistance. CYP1A2 polymorphism is correlated to increased risk of colon cancer when it is activated by carcinogenic components such as tobacco smoking.[2] The absent CYP2E1 expressions in colon may become important factor because it may suggest that the organ tends to turn into cancer.[7] CYP2E1 enzyme is strongly correlated to the incidence of gastric cancer, and naturally the enzyme activity is induced by alcohol, tobacco smoking and other endogenous gastric factors. Nishimoto et al, studied about CYP2E1 polymorphism on gastric cancer and found that the CYP2E1 genotype variant is correlated to reduced cancer risk of upper gastrointestinal tract. However, the correlation between CYP2E1 polymorphism and gastrointestinal cancer is still controversial up to now.[6]

CONCLUSION

Gastrointestinal mucosa is the first location where oral xenobiotics undergo biotransformation process through the aid of CYP450 enzymes. Subfamily 1, 2, and 3 of CYP450 are the most important enzyme in oral drug metabolism. Polymorphism of CYP450 enzymes in gastrointestinal mucosa affects the biotransformation activity of oral drugs (poor metabolizers, extensive metabolizers or ultra rapid metabolizers). The clinical consequences will affect good therapeutic response. Therefore, we should be careful in determining the effective (individual) dose as well as avoiding multiple drugs therapy in order to reduce the drug effect and oral multidrugs resistance. Polymorphism of CYP450 enzyme induced by alcohol, tobacco smoking or endogenous gastro-intestinal factor will activate carcinogenic factors in the gastrointestinal tract.

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