Review Article

Species differences on Intestinal CYP Expression

Abstract

The CYP450 enzyme family is most responsible for the biotransformation of endobiotic and exobiotic. Eventually, this family will consist of the most abundant drug-metabolizing enzymes. Though the liver is the major metabolizing organ for drugs and xenobiotics, orally administered drugs can be pre-metabolized before reaching systemic circulation. This metabolism takes place in the small intestine, which is the second-largest metabolizing organ after the liver. Because intestinal cells from various laboratory animals can be examined to predict the pre-systemic metabolism of orally administered drugs, this study hopes to shed light on species differences in intestinal gene expression. The most abundant human CYP subfamilies were CYP3A, and the most highly expressed isoform was CYP3A4, which was completely mimicked by monkeys' intestinal CYP expression. CYP1A1 was expressed in all species' intestines except dogs. Other commonly expressed isoforms for all species were CYP1B1, CYP2E1, CYP2J2, and CYP3A5. Humans, mice, and monkeys have shown the most similarities. The homologies of CYP1A1 and CYP1A2 were found to be identical in monkeys, but substrate specificity varied. Humans have interindividual variation in the expression of enzymes, which is not an attributing problem for other species. In all the species studied, the distribution of enzymes varies with the different segments of the intestine, and the level of protein expression also varies while reaching distal regions. Further summarization of CYP expression in different segments of the small intestines of humans will help in pharmacokinetic studies.

Keywords: CYP450, small intestine, liver, human, monkey, mouse, pharmacokinetics, CYP3A4.

1. Introduction:

Molecules inside the body, either endobiotic or exobiotic, are bio-transformed by the activity of cytochrome P450 enzymes (CYPs) [1-6]. These highly responsible enzymes are membrane bound enzymes and they are known to have wider binding pockets to accommodate a broader range of substrates [1, 7-10].These enzymes are evidently most important drug-metabolizing enzymes (DMEs). Being most important DMEs and possessing diverse substrate specificity makes them imperative in metabolic drug-drug interactions (DDIs) because multiple drug molecules can bind or compete for the same or different active sites in the same enzyme[4, 11-14]. National Cancer Institute (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cytochrome-p450-enzyme-system) defines cytochrome P450 enzyme system as "*A group of enzymes involved in drug metabolism and found in high levels in the liver. These enzymes change many drugs, including anticancer drugs, into less toxic forms that are easier for the body to excrete.*" As the name implies, there are mainly three parts in this name: a. "cyto" which emphasizes that they are bound to membranes within the cell; b. "chrome and P" which implicate colorful heme pigments, and c. 450 means that these proteins produce a spectrum with an absorbance maximum at 450 nm when it bounds to carbon mono-oxide (CO). The nomenclature of these enzymes is done using prefix CYP followed by family number, subfamily letter, and isoform number. Within a family there are more than 40% homology, within subfamily there are more than 55% homology in amino acid[15, 16]. For example, for CYP2C19 isoform, it will have more than 40% genetic similarity with another CYP2 enzymes including CYP2B, and it will have more than 55% match with other CYP2C including CYP2C8. Only for human CYPs, capital letters are used (for example CYP3A4 or CYP1A1), for rodents only first letter capitalized (for instance Cyp1a1 and Cyp3a1). Numerous CYP proteins and more than 57 functional genes have been discovered during the past 80 years; they have been divided into various families and subfamilies.[3, 17, 18].

CYP enzymes, also known as cytochrome P450 enzymes, are a large family of enzymes that are found in a wide variety of organisms, including bacteria, plants, and animals[1, 16, 19, 20]. These enzymes are important for a number of bioconversions, both endobiotic and exobiotic.Endobiotic bioconversions refer to the metabolism of compounds that are produced within an organism. CYP enzymes play a crucial role in the metabolism of drugs and other xenobiotics (foreign compounds) within the body. They are responsible for the metabolism of a wide variety of compounds including drugs, hormones, and toxins. They are also responsible for the metabolism of endogenous compounds, such as cholesterol and fatty acids.Exobiotic bioconversions refer to the metabolism of compounds that are not produced within the organism[3]. Many microorganisms, such as bacteria and fungi, use CYP enzymes to degrade and detoxify environmental pollutants. These enzymes can also be used to activate prodrugs (inactive compounds that are converted into active drugs once inside the body) and to synthesize new compounds, such as pigments and flavors.CYP enzymes are important for maintaining the balance of chemicals within the body and for protecting the organism from harmful compounds. Because of their key role in metabolism, CYP enzymes are also the target of many drugs and toxins.Overall, CYP enzymes play a crucial role in the bioconversion of a wide variety of compounds, both endobiotic and exobiotic, and are important for maintaining the health and well-being of organisms.

Metabolism is the set of chemical reactions that occur within an organism to maintain life[3, 21-24]. These reactions take place in a variety of organs throughout the body. The liver is the main

organ involved in metabolism[25-35]. It is responsible for detoxifying harmful compounds, such as drugs and toxins, and for the metabolism of nutrients, such as carbohydrates, proteins, and fats. It also plays a key role in the regulation of blood sugar levels.The kidneys are responsible for regulating the balance of electrolytes, such as sodium and potassium, in the body and for filtering waste products from the blood. Skeletal muscles are responsible for the metabolism of glucose and fatty acids during exercise.Adipose tissue, also known as body fat, is responsible for the storage and release of energy in the form of fatty acids.The pancreas is responsible for the production of insulin and other hormones that regulate blood sugar levels.The intestines play a role in the absorption and metabolism of nutrients from food[7, 13, 14, 22, 34, 36-38].The heart is responsible for the metabolism of fatty acids and glucose during physical activity. The lungs are responsible for the metabolism of oxygen and the elimination of carbon dioxide.Overall, the liver is considered the main organ involved in metabolism, but all the mentioned organs play a significant role in different metabolic processes.

First-pass metabolism, also known as first-pass effect or pre-systemic metabolism, refers to the process by which a drug or other compound is metabolized before it reaches the systemic circulation[12-14, 34, 37-41]. This occurs when a compound is absorbed through the gut and then enters the liver through the portal vein, where it is metabolized by enzymes such as cytochrome P450 (CYP) enzymes, in the liver.The first-pass metabolism can have a significant impact on the bioavailability of a drug or other compound, which is the amount of the compound that reaches the systemic circulation and is available to produce a therapeutic effect. Because the liver is the main site of first-pass metabolism, drugs that are metabolized extensively in the liver will have a lower bioavailability than those that are metabolized to a lesser extent.For example, oral administration of a drug, the drug must pass through the gut and liver before it reaches the systemic circulation. If a large amount of the drug is metabolized in the liver, only a small amount will reach the systemic circulation and produce a therapeutic effect.

This is important to consider in the development of new drugs, as compounds that are metabolized extensively in the liver may not be effective when given orally and alternative administration routes, such as intravenous or transdermal, may be needed. Generally, first-pass metabolism is a critical process that can have a significant impact on the bioavailability and effectiveness of drugs and other compounds, and it is an important factor to consider in the development of new medications.

The intestine plays a role in first-pass metabolism by absorbing drugs and other compounds from the gut and transporting them to the liver through the portal vein. The intestine plays a key role in the absorption of drugs that are administered orally, as it is responsible for the transport of drugs across the gut wall and into the bloodstream[10, 12, 14, 22, 42, 43].The gut wall contains a number of enzymes, transporters, and efflux pumps that can also contribute to first-pass metabolism. For example, enzymes such as carboxylesterases and sulfatases can metabolize drugs before they reach the liver. Transporters such as P-glycoprotein can pump drugs out of the gut wall and back into the gut lumen, reducing the amount of drug that reaches the liver.Additionally, the gut microbiome (microorganisms that live in the intestine) can also play a role in first-pass metabolism by metabolizing drugs and other compounds[4, 13, 14, 27, 41, 44-47]. Some microorganisms can produce enzymes that can metabolize drugs, and some can even produce new compounds that can interact with drugs.Overall, the intestine plays a key role in the absorption of drugs and other compounds from the gut and their transport to the liver, but it also contains enzymes, transporters, and efflux pumps that can contribute to first-pass metabolism

and reduce the bioavailability of drugs. Additionally, gut microbiome plays an important role in first-pass metabolism by metabolizing drugs and other compounds.

Preclinical studies are conducted in animals before a drug or other compound is tested in humans. A number of different species are used in preclinical research, depending on the type of study and the research question. The most common preclinical species used are:

i. Rodents: Mice and rats are the most commonly used species in preclinical research. They are small, easy to handle, and have a short lifespan, which makes them useful for studies of short-term effects. They are also genetically and physiologically similar to humans, which makes them useful models for studying human diseases.
ii. Non-human primates (NHPs): NHPs such as monkeys and apes are used in preclinical studies when a more accurate model of human physiology, pharmacology and toxicology is needed. They are more expensive and time-consuming to use than rodents, but they are the best model to predict human response.
iii. Canines: Dogs are used in preclinical research to study diseases of the cardiovascular and respiratory systems, as well as certain metabolic and endocrine disorders, that are similar to those found in humans.
iv. Pigs: Pigs are used in preclinical research to study diseases of the cardiovascular and respiratory systems, as well as certain metabolic and endocrine disorders. They are also used for preclinical studies of xenotransplantation, which is the transplantation of organs or tissues from one species to another.
v. Zebrafish: Zebrafish are increasingly used in preclinical research as they are easy to maintain, reproduce quickly and genetically manipulated. They are also transparent when they are young, which allows for in vivo imaging.

These are some of the major preclinical species that are commonly used in preclinical research, but other species such as birds, fish, amphibians and invertebrates are also used depending on the specific research question.

Preclinical species, such as mice and rats, are used in drug development because they share many physiological and genetic similarities with humans. Testing new drugs on these species allows researchers to gain insight into potential efficacy and side effects before conducting clinical trials on humans. Additionally, preclinical testing in animals can help identify potential safety concerns early in the drug development process, reducing the risk of harm to human subjects.

Species-specific knowledge is important in drug discovery and development research because different species can respond differently to drugs. For example, a drug that is effective in humans may not be effective in mice or may even cause harmful side effects. Understanding the unique physiology, physiology and biochemistry of a species is crucial to ensure that drugs are safe and effective for that species. Additionally, species-specific knowledge allows researchers to optimize dosing and administration methods for a specific species, which can improve the accuracy and reliability of preclinical data. This knowledge also provides insight into the possible mechanism of action of a drug and the targets in the body that are affected. Therefore,

species-specific knowledge can help speed up the drug development process by providing a clearer understanding of the drug's effects and increasing the chances of success in clinical trials.

Similarly, the CYP enzyme expression in different preclinical species is different than that in human. The aim of this review is to gather current knowledge on intestinal Cytochrome P450 enzyme expressions in different species including humans.Recently published research articles on intestinal CYP enzyme expressions in different species were searched using relevant and specific key words [48].

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2. Species differences of intestinal CYP expression

a. Mouse

Until now, only a limited number of studies have been conducted to understand the expression of CYP enzymes in mice. These studies have used various methods such as 7,12-dimethyl[α]anthracene hydroxylation, benzo[α]pyrene hydroxylation, phenobarbital-inducible coumarin dealkylation, aniline and biphenyl hydroxylations, and ethylmorphine N-demethylation to analyze CYP expression in mouse enterocytes. Another study utilized a polychlorinated biphenyl mixture and western blotting to examine the small intestine of aryl hydrocarbon receptor-positive mice. However, the inconsistent results from different mouse strains and isolation techniques prompted a new study.

The new study aimed to overcome these inconsistencies by using qualitative RNA-PCR, real-time quantitative RNA-PCR, and immunoblot analysis to determine the expression level and inductive nature of CYPs in the mouse intestine. The results showed that CYP1A1 expression was low, while CYP2B, CYP2C, and CYP3A were abundant in the small intestine. This was based on the immunoblot analysis of mice induced with beta napthoflavone (BNF), while the RNA-PCR of untreated mice showed a more accurate result. The expressions of CYP1A1, CYP1B1, CYP2B10, CYP2B19, CYP2B20, CYP2C29, CYP2C38, CYP2C40, CYP2E1, CYP3A11, CYP3A13, CYP3A16, CYP3A25, and CYP3A44 were found to be in appropriate amounts, with CYP1A1 and CYP2E1 showing lower expression.

Additionally, the expression of these enzymes was found to be higher in the proximal end of the small intestine compared to the distal end. As the distance along the small intestine of mice was traveled, the expression of CYP2B, CYP2C, and CYP3A was found to diminish. These results provide new insights into the expression of CYP enzymes in mice, and could have important implications for understanding the metabolic processes in this species [49].

b. Rat

A study was conducted to evaluate the expression of CYP enzymes in the epithelial cells of rat intestine using RNA-polymerase chain reaction and immunoblot techniques. The results showed that CYP expression was different and more restricted in the intestine compared to the liver. The rats were treated with and without beta-naphthoflavone (BNF), phenobarbital, and pregnenolone-16alpha-carbonitrile or dexamethasone. In both groups, sufficient expression of CYP1A1, CYP2B1, and CYP3A1 mRNA was observed for translation. However, expressions of CYP2A1, CYP2B2, CYP2E1, CYP3A2, and CYP4A1 were found in hepatic cells, but no or negligible expression was detected in the rat intestine.

The most induced isoform in the rat intestine was CYP1A1, but it was found to be affected by the route of administration of the inducers. These results highlight the differences in CYP expression between the liver and intestine and could have important implications for understanding the metabolic processes in rats. This information can also be useful in the design of drug treatment strategies and the development of new drugs [50].

c. Pig

For studying metabolism pattern of different drugs in the intestine, pig or porcine can be another option along with other common study animals as rat & mouse. Pig possesses similar physiological and anatomical pattern mostly in digestive and cardiovascular system.During study of expression of CYPs in the enterocytes of pigs, it has been observed that intestinal cells lose the viability and cellular differentiation pattern very rapidly. Western blotting technique were applied,and it confirmed the expression of CYP1A and CYP3A enzymes in the porcine enterocytes.Along with that, the induction of CYP1A enzymes including CYP1A1 & CYP1A2 were also observed in enterocytes [51]. Another comparative study has been done on human jejunal tissue and pig jejunal tissue, which revealed the common CYP subfamilies between these two are CYP1A, CYP2C, CYP2D, CYP2E, CYP2J, CYP3A. Enzymes of these subfamilies showed identical amino acids homology in greater percentage. Pig intestinal CYP3A46 showed 77% similarity with human CYP3A4. CYP2E1 and CYP1A1 in both samples showed identical homology as 80% and 82 % respectively. CYP3A46 showed the highest expression in pig jejunum tissue whereas highest expressed CYP3A4 of human. In the order of descending, CYP2C42, CYP1A, CYP2D25 were followingly expressed,and the least were CYP2C49[52].

d. Dog

Beagle dogs were used to interpret the results of oral pharmacokinetics during the development and formulation of drugs for human use. For analyzing the expression of CYP enzymes in the dog's intestine, mass spectroscopy was used. Mass spectroscopy is a quantitative method for determining the abundance of drug metabolizing enzymes in animal intestines. Intestinal tissue of both male and female dogs was examined, and various approaches were taken to reduce the changes due to longer tissue handling times. One of the important methodologies was to introduce protease inhibitors to minimize intestinal protein degradation. The enzymes that were used for quantification were CYP1A2, CYP2B11, CYP2C21, CYP2D15, CYP2E1, CYP3A12, and CYP3A26. CYP2B11 and CYP3A12 were discovered in the intestine, but in lower concentrations than in the liver. CYP3A12 in the intestine was more expressive than CYP3A26 in the liver. Another study suggested that intestinal enzymes are more vulnerable to losing activity during processing, mostly intestinal CYP2B11, and that for that reason they show less expression than in the liver. CYP1A1 and CYP1A2 were not found to be expressed in the dogs' intestines constitutively[53].

In another advanced study that was aimed at identifying scaling factors for metabolism, they worked on finding out the expression of CYPs in dogs' intestines. The same mass spectroscopy method has been used to quantify seven CYP enzymes. The difference in enzyme activity in the intestine is not clear, as the enzyme activity can be decreased during processing. According to their findings, CYP3A12 was the most abundantly expressed CYP enzyme in the intestine, followed by CYP2B11. Activity of enzymes decreased (using midazolam) when traveling from the small intestine to the colon, and the quantity also declined for CYP3A12 and CYP2B11 in the distal intestine [54].

e. Monkey

Cynomolgus monkeys were chosen to study human metabolism patterns because, among other experimental species, monkeys have the same evolutionary similarities as humans. Studies were carried out using the small intestine and found variation in the distribution of CYP enzymes in various parts of the intestine. Activity was measured using midazolam, 7-ethoxyresorfin, coumarin, paclitaxel, diclofenac, *S*-mephenytoin, testosterone, tolbutamide, bufuralol, and chlorzoxazone as substrates on seven human CYPs. Epithelial cells of the duodenum and cells from other locations in the jejunum and ileum were processed for analysis. The reaction rate was found higher in the jejunum than the ileum, which indicates the uneven distribution of CYPs throughout the intestines of monkeys. The reactivity of CYP3A4 and CYP3A5 was found to be higher in the jejunum due to the higher expression of CYP3A in this area. CYP2C75, CYP2C43, CYP2C76, CYP1A1 and CYP1A2 are among the enzymes whose expression levels are lower in the ileum than in the jejunum. Among these, CYP1A1 and CYP1A2 are highly identical with human CYP1A1 and CYP1A2. Another enzyme from monkeys, CYP2D17, was discovered to be identical to human CYP2D6. Additionally, mRNA expression of CYP3A5 has been found to be abundant in the intestines (distal ileum). Another study has also been done that examined extensively the mRNA expression of 14 CYP enzymes in the intestine of a cynomolgus monkey using RT-PCR. Except CYP1A2, all other enzymes, including CYP1A1, CYP2A23, CYP2A24, CYP2B6, CYP2C20, CPY2C43, CYP2C75, CYP2C76, CYP2D17, CYP2E1, CYP2J2, CYP3A4, and CYP3A5, were detected in the small intestines. Similar to the previously stated result, again the most abundantly expressed enzyme was CYP3A4 in the jejunum, followed by CYP3A5, CYP2C75, CYP2J2, CYP2D17, CYP2C43, CYP1A1, CYP2A23/CYP2C76, CYP2B6, CYP2A24, CYP2C20, and CYP2E1. Among the most abundant CYP2Cs was CYP2C75, which was discovered to be homologous to human CYP2C9 and CYP2C19 [55, 56]. Another study was specifically designed to investigate the similarity in the homology of intestinal CYP450s between humans and monkeys. The identity has been found to be 90% identical but with substrate and inhibitor specificities. Diclofenac 4'-hydroxylation is triggered more by CYP2C19 than CYP2C9, but in humans it is an indicator reaction of CYP2C9. Another example of substrate specificity is Cynomolgus CYP2C76, which is not expressed in humans but is identical to human CYP2C subfamilies and performs non-CYP2C substrate metabolism. All of these are responsible for species differences [57].

f. Human

The main drug metabolizing enzymes in humans are CYP3A and CYP2Cs. Enzymes of the CYP3A subfamily are responsible for 80% of metabolism, while CYP2C does 18%. Another enzyme that is constantly present is CYP2J2. Expression of CYP1A1 varies between individuals. CYP2D6 is expressed, but it has a polymorphic nature, and the major hepatic CYP2E isoforms were found to be poorly expressed in the intestine. CYP circulation varies throughout the small intestine and major distribution was found in the proximal regions, as with other species [58]. Another study that has been done on human enterocytes using EDTA-buffer-mediated elusion and analyzed by reverse transcriptase-polymerase chain reaction The results showed that the mRNA of CYP1A1, CYP1B1, CYP2C, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 enzymes is expressed in the human intestines. The detection of CYP2C and CYP3A4 was only done by western blotting, and expression of CYP1A1 was experienced in two of the eight intestines. The activity of these enzymes, including CYP3A4, increased on moving to the jejunum from the duodenum and again decreased in the direction of the ileum, but levels of enzymes remained

constant. Among all tested CYPs, only CYP3A5 was not detected in the human intestine samples [59]. After CYP3A4, the most significantly expressed enzyme was CYP2C. In another advanced study, the interindividual variations in expression were evaluated by analyzing metabolic activities quantitatively. Inter-individual variations in terms of metabolic activity of CYP3A4, CYP2C9, and CYP2C19 were confirmed, with poor correlation among these major intestinal metabolic enzymes. Though CYP2C19 and CYP3A4 were significant but poorly correlated, the correlation between CYP2C9 and CYP3A4 was absent [60]. A mass spectroscopy-based study aimed to quantify protein expression in the different segments of the small intestine for the 16 majors human CYP isoforms. Among these, CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP3A4, CYP3A5, and CYP4F2 were detected, and quantified, and substantial inter-individual variability was found for CYP2D6. Though CYP3A5 was not detected in a previous study, protein activity for this isoform was observed to increase from the duodenum to the ileum in this study. CYP2J2 followed the same trend, while CYP3A4 was more highly expressed in the jejunum than the ileum. Duodenal expression of CYP4F2 was found lowest in comparison to the jejunum and ileum. This study reveals that correlation among isoforms has been more profound when quantifying protein expression than mRNA expression [61].

3. Summary and Conclusions

The researchers utilized various techniques such as western blotting, immunoblotting, reverse transcriptase polymerase chain reaction, real-time polymerase chain reaction, mass spectroscopy, and liquid chromatography to analyze the CYP expression in the epithelial cells of the rat intestine. The results showed that the CYP expressions in the intestine were different and more restricted compared to the liver. Rats were treated with and without inducers such as beta-naphthoflavone, phenobarbital, and pregnenolone-16alpha-carbonitrile or dexamethasone. In both groups, enough CYP1A1, CYP2B1, and CYP3A1 mRNA was present for translation. However, CYP2A1, CYP2B2, CYP2E1, CYP3A2, and CYP4A1 were found to be present in the hepatic cells but not in the rat intestine. The most induced isoform in the rat intestine was CYP1A1, but this can be affected by the route of administration of the inducers.

The CYP subfamilies that were expressed among all species were CYP1A, CYP2B, CYP2C, and CYP3A. The human intestinal metabolic enzymes were CYP1A, CYP2C, CYP2D, CYP2E, CYP2J, and CYP3A, and there were similarities with other species' subfamilies. The isoforms of CYP3A subfamilies were highly expressed among these. CYP1A1, CYP1B1, CYP2E1, CYP1A2, CYP3A4, CYP3A5, CYP2J2 were found to be expressed in more than one species, including the human intestine. CYP1A1 was present in all species except dogs, while CYP2E1 was absent in rat intestines. CYP1A2 was found to be expressive in pigs, monkeys, and humans, but absent in dogs. The amino-acid sequences of CYP enzymes from pigs and monkeys were found to be identical to human CYP homology. The monkey CYP1A1 and CYP1A2 were identical to human CYP1A1 and CYP1A2; CYP2D17 was homologous to human CYP2D6; and CYP2C75 was homologous to human CYP2C9 and CYP2C19. The researchers found that the substrate and inhibitor specificity of these homologous enzymes from monkey and human were different, and there was an attribution problem in quantifying human CYPs' proteins and mRNA expressions due to interindividual variation and polymorphism (CYP2D6). CYP3A4 was the most abundantly expressed enzyme in both humans and monkeys. This data suggests that monkeys' intestinal cells could be used to evaluate the pharmacokinetic profile of orally

administered drugs. The mouse also showed similar patterns in isoform expressions to humans, making it the best choice for research. However, the patterns of dogs' intestinal enzyme expressions were found to be less promising.

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