CHAPTER 11

The CYP3 Family

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Table of Contents

11.1 Introduction

The CYP3A enzymes are of major importance in human biology and clinical therapeutics.[1,2] They are the most of abundant of the CYP enzymes in the

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liver,[3] and are the only CYPs present in substantive amounts in the enteric mucosa of the gastrointestinal tract. Substrate specificity is broad; CYP3A mediates the biotransformation of numerous endogenous substances, environmental chemicals of potential toxicological relevance, and medications used in clinical therapeutics (Table 11.1).

The significance of CYP3A in terms of human evolution and species preservation is a topic of logical speculation. The lack of a phenotypic "null" status for CYP3A – corresponding for example to CYP2D6 "poor metabolisers" – supports the notion that CYP3A is essential. Homeostasis of many endogenous steroid hormones, including those required for sexual maturation and reproduction, is dependent on CYP3A.[4,5] A number of environmental chemicals, both therapeutic and toxic, derived from plants and fungi are in fact substrates for metabolism by CYP3A enzymes. Examples include the cinchona alkaloids, the taxanes, opiates, aflatoxins, and the immunosuppressants cyclosporine, tacrolimus and sirolimus. Further clues derive from the anatomic distribution of human CYP3A, and its unique kinetic characteristics. The dual localisation of CYP3A in enteric mucosa and liver provides the species with "two shots" at protection against potentially toxic environmental chemicals before they reach the systemic circulation (Figure 11.1). The property of binding cooperativity,

Table 11.1 Representative CYP3A substrate drugs used in clinical practice.

Clearance completely or nearly completely dependent on CYP3A	*Clearance partially dependent on CYP3A*
Alfentanyl	Amitriptyline
Alprazolam	Citalopram
Atorvastatin	Clozapine
Buspirone	Dextromethorphan
Carbamazepine	Diazepam
Cyclosporine	Imipramine
Eletriptan	Methadone
Erythromycin	Omeprazole
Felodipine	Sertraline
Midazolam	Telithromycin
Nefazodone	Voriconazole
Nifedipine	Zolpidem
Quetiapine	
Quinidine	
Ritonavir	
Saquinavir	
Sildenafil	
Simvastatin	
Tacrolimus	
Tadalafil	
Trazodone	
Triazolam	
Vardenafil	
Verapamil	

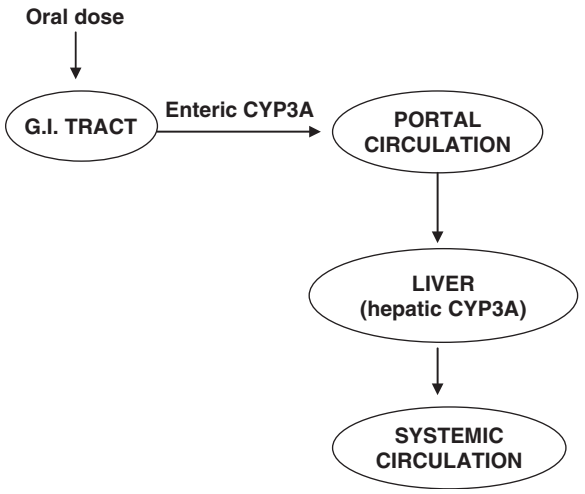

Figure 11.1 Schematic diagram of the fate of an orally-administered CYP3A substrate drug prior to reaching the systemic circulation. Metabolism by CYP3A4 and CYP3A5 is possible during passage across the enteric mucosa of the proximal small bowel as well as during passage through the liver.

yielding homotropic autoactivation, is consistent with a protective effect of increased CYP3A activity upon exposure to high and potentially toxic quantities of exogenous substrate.[6] Finally, there is extensive (but not complete) overlap in affinity of substrates for metabolism by CYP3A along with efflux transport by P-glycoprotein.[7–9] Many of the natural substances described above are substrates for both, consistent with a coincident "protective" purpose.

A notable observation not supporting the central importance of CYP3A is that no life-threatening medical consequences are known to arise from extended treatment with highly potent CYP3A inhibitors such as ketoconazole[10] and ritonavir.[11] Both of these drugs produce what amounts to a chemically-induced "poor metaboliser" phenotype. The antifungal agent ketoconazole has been available since the early 1980s, and the viral protease inhibitor ritonavir since the mid 1990s. Extended exposure to ketoconazole is associated with antiandrogenic effects, and a lipodystrophy syndrome has been linked to extended treatment with ritonavir and other protease inhibitors.[12] These sequelae are possibly attributable to CYP3A inhibition, but this is not proven. In any case, the sequelae are significant but not ominous medical consequences, raising questions as to how essential it is in adults for CYP3A phenotype to be maintained within a "normal" range.

The unique protective function of hepatic and enteric CYP3A enzymes may produce complications in drug development and clinical therapeutics. New chemical entities found to be complete or nearly complete substrates for clearance by CYP3A may actually be dropped from subsequent development. Such candidates often are seen as facing therapeutic or competitive

agents that are CYP3A inducers or inhibitors, and restrictive labelling that could result. Some marketed drugs, such as terfenadine, astemizole and cisapride were actually withdrawn for that reason.[13–16] Similar concerns apply to new drug candidates that themselves are found to be significant CYP3A inducers or inhibitors. Some CYP3A substrate drugs that meet a pressing medical need, such as the viral protease inhibitors saquinavir and lopinavir, have been brought through the development process and approved for clinical use despite the drawbacks and obstacles. These two drugs have the disadvantage of poor net oral bioavailability, due to some combination of hepatic/enteric presystemic extraction together with enteric efflux transport by P-glycoprotein (P-gp). So significant is this problem that saquinavir and lopinavir are, with few exceptions, combined with the CYP3A/P-gp inhibitor ritonavir for purposes of ''boosting'' or ''augmentation'' of oral bioavailability.[17–19]

The topic of this review is the translational pharmacology of CYP3A enzymes in humans. The principal focus will be a number of contemporary and/or controversial issues of current importance.

11.2 Pharmacogenomics

The four genes encoding the relevant CYP3A proteins (CYP3A4, 5, 7, and 43) are all located in a 231-kb cluster on chromosome band 7q21-q22.1. The relationship of CYP3A genomic variants to CYP3A protein expression and activity *in vitro* and *in vivo* has been extensively investigated over the last decade.[20–25] The prevailing contemporary interpretation of the existing data base is that a great deal of information has been generated, collectively demonstrating very little in the way of meaningful associations between CYP3A genotype and *in vivo* phenotypic metabolic activity. Furthermore, there is substantial disconnect between *in vitro* studies and human pharmacokinetic studies *in vivo*.

CYP3A7 is a foetal enzyme, and its expression is silenced after birth. Reports exist of persistently detectable CYP3A7 mRNA in adulthood, but there is no available evidence that CYP3A7 is of any significance in terms of *in vivo* drug-metabolising activity. CYP3A43 is an adult enzyme, and is known to be localised in prostate.[26,27] There is no known functional significance of CYP3A43 identified to date.

CYP3A4 is the dominant isoform in humans. Numerous studies have demonstrated that CYP3A4 is the most abundant of the human hepatic cytochromes P450 and also is (along with CYP3A5) the only cytochrome P450 of functional importance in the enteric mucosa of the gastrointestinal tract. Expression of hepatic and enteric CYP3A4 is not coordinately regulated; levels expressed at the two sites are not intercorrelated.[28]

Many single nucleotide polymorphisms (SNPs) have been identified in the CYP3A4 locus.[29–32] Some of these SNPs are associated with reduced functional

substantial evidence that any CYP3A4 SNP is associated with clinically important differences in clearance of CYP3A substrates *in vivo*.[31,34–42] Consistent with this is the observation that phenotypic distribution of CYP3A metabolic activity *in vitro* and *in vivo* is unimodal rather than multimodal (bimodal or trimodal),[43–46] thereby essentially excluding the existence of common ''null'' alleles coding for low or zero protein expression or function. Finally, all CYP3A4 SNPs with demonstrated functional consequences are of low prevalence in the population. Nonetheless it cannot be fully excluded that one or more SNPs (such as CYP3A4*20)[33] could account for low CYP3A metabolic activity noted in some unusual ''outlying'' subjects in a number of human studies.[46–48]

The CYP3A4*1B polymorphism is located in the 5′-regulatory region of the *CYP3A4* gene. This SNP received considerable attention when it was first described, due to the statistical association with poor outcome in patients with prostate cancer.[49] A link of CYP3A4*1B to altered testosterone metabolism was speculated as possible mechanism, but the study did not evaluate plasma testosterone or any other index of metabolic activity. A few subsequent reports provided some evidence supporting associations of the CYP3A4*1B polymorphism with human disease, but other studies have not.[50–57] In careful clinical phenotype-genotype studies using CYP3A probe substrates such as midazolam, a detectable phenotypic importance of CYP3A4*1B was not found.[31,34–40,58] Thus the mechanism of the linkage of the CYP3A4*1B polymorphism to human disease, if a link actually exists, remains unexplained. It has been postulated that a partial linkage disequilibrium of CYP3A4*1B with CYP3A5*3 may explain the association, inasmuch as CYP3A5 is found in prostate and may play a role in local androgen metabolism.[59] However this is a speculative explanation without experimental support.

CYP3A5 shares approximately 90% sequence homology with CYP3A4. CYP3A5 is expressed in liver and gastrointestinal enteric mucosa, as well as a number of other tissues including prostate and kidney.[60] In contrast to CYP3A4, there is evidence that CYP3A5 is polymorphically expressed.[59–63] The CYP3A5*3 variant, which is the most prevalent form in many human populations, actually confers low or zero functional CYP3A phenotypic activity, whereas the less prevalent CYP3A5*1 codes for active protein. Expression of immunoactive CYP3A5 protein in human liver samples is consistent with genotype. Since CYP3A5*1 has greater prevalence in the African-American population compared with Caucasians, the possibility is raised – politically alluring to some – that a racial difference in CYP3A phenotype may exist. This has been supported by *in vitro* studies of CYP3A metabolic activity in genotyped human liver samples. However, the outcomes of clinical studies are not consistent with this scheme. Human CYP3A metabolic phenotype is, at most, weakly associated with CYP3A5 genotype, and there is little or no evidence of a race-associated difference in metabolic activity.[31,34–36,41,64–66] Again this demonstrates the *in vitro-in vivo* disconnect described previously. At present, the abundance of evidence indicates that CYP3A5 and its

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