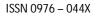**Review Article**

Cytochrome P450: Nature's Untapped Superpower

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ABSTRACT

Cytochrome P450 are a superfamily of hemeproteins present in many living organisms including man, playing important roles in physiological and pathological processes. Ever since their discovery in the early 1950's research has been a never ending process with several cytochrome functions not being known yet. This field poses potential for future drug development boosting the concept of "personalized medicine". This review aims at providing a unique summary of all the research that has gone into this enzyme system to provide new dimensions to further research and development of drugs.

Keywords: Cytochrome P450, enzyme induction, single nucleotide polymorphism, xenobiotics.

INTRODUCTION

As early as in the 1950's, Brodie and Miller demonstrated the capacity of mammalian tissues to oxidize nonpolar xenobiotics. Dr.Julius Axelford and his colleagues refined Brodie's work and hypothesized that this metabolic function was of an enzyme system particularly in the mammalian liver. In 1958, Dr. Martin Klingenberg went a step further and demonstrated the presence of a CO-binding pigment in liver microsomes with an absorption maximum at 450 nm. Finally, Ryo Sato and Tsuneo Omura successfully identified it as a P450 Hemeprotein with the unique spectral peak, thought to result from the *trans orientation* between the cysteinate and carbon monoxide ligands of the enzyme's associated heme group and named it Cytochrome P450.[1] Thus, came into being the world's most universal biocatalyst.

A look at the structure of these enzymes reveal that they are a superfamily of proteins with heme as a cofactor non covalently linked to a polypeptide chain. The illustration of the 3D structure revealed that the majority of the structure is made up of an alpha-helical arrangement, but N- and C-terminal small beta-domains can be found .The size and shape of heme pocket determines the different isoforms of CYP.

The various CYP Hemeproteins are divided into gene families and subfamilies based on the sequence homology (more than 40% and 55% respectively). 18,000 CYP's have been identified which are present in animals, plants, fungi, archae, protists, viruses and bacteria (except *E.coli*). In man alone, 18 families and 43 subfamilies are present. Of these, majority of the enzymes are localized in the smooth endoplasmic reticulum primarily in the liver, intestine, lung, kidney and placenta.

Cytochrome P450 is a group of monooxygenases that catalyze oxidation of organic compounds that accounts for 70 % of drug metabolism. They also play a central role in steroid hormone and vitamin metabolism. P450s also play a major role in plants by catalyzing the synthesis of a large number of secondary metabolites. Apart from these, there exist few orphan cytochromes whose function is yet to be elucidated.

The most common CYP enzyme involved in Phase I metabolism is CYP 3A4 which is absent in the fetus, appearing only at the 4th month after birth increasing to its adult quantity by 1 year of age and has the unique features of a large active site and ability to bind to more than one substrate using weak Van der Waal forces and accounts for 31.4% of drug metabolism. The other important drug metabolizing enzymes are CYP 2D6, CYP 2C9 and CYP1A2 accounting for 30%, 10% and 2% of the drug metabolism respectively with the other enzymes cumulatively accounting for 3 % of the drug metabolism. The decision as to which CYP enzyme would metabolise the substrate from the huge array of CYP enzymes is based on the volume, pKa and planarity of the substrate.[2]

Apart from the large number of substrates metabolized by the CYP enzymes, various other agents serve as inducers and inhibitors and either increase or decrease the activity of the enzyme respectively and thus account for drug-drug interactions some of the important ones from clinical point of view being elucidated as follows.

Inhibition

One drug inhibits the metabolism of another drug with resultant increase in the circulating levels of the slowly metabolised drug and prolongation of potentiation of its pharmacological effects. There are three basic types of enzyme inhibition (competitive, non-competitive and uncompetitive).

Competitive inhibition occurs between the inhibitor and substrate for the same binding site on an enzyme. Some examples being omeprazole and diazepam (CYP2C19), metronidazole and warfarin (CYP 2C9), diltiazem and prednisone (CYP3A4).[3]

Non competitive inhibition involves an inhibitor binding at a site on the enzyme distinct from the substrate thus altering the confirmation of the enzyme for example, combined administration of cimetidine and CYP 3A4 substrates.

This phenomenon of enzyme inhibition has been utilized for therapeutic effect i.e. in alcohol aversion therapy but may also result in serious adverse events such as enhanced bleeding tendency with dicumarol when given along with cimetidine.

Induction

Several drugs on repeated administration induce the growth of smooth endoplasmic reticulum and thus enhance the microsomal enzyme activity. Similar to inhibition this may be used for therapeutic benefit such as use of phenobarbitone a week to fortnight prior to labour for treatment of neonatal jaundice or may also result in adverse effects if not thought of at time of administration of multiple drugs concomitantly.

Despite all the various factors that may influence the CYP metabolism the most important factor accounting for the variability in CYP functions are the various genetic polymorphisms, the most common being Single nucleotide polymorphisms accounting for 0.1% of all genetic polymorphisms. As a result of the differences in the DNA sequences, three types of metabolisers are categorized: *extensive metabolizer* (normal), *poor metabolizer* (decreased metabolism), *ultra-rapid metabolizer* (increased metabolism of xenobiotics) described for the first time after observing effects of CYP2D6 genetic polymorphisms on metabolism of dibrisoquine and sparteine.Since then genetic polymorphisms have been demonstrated in all CYPs the most important being CYP2C9 (clearance of warfarin), CYP2C19 (hydroxylation of S-mephenytoin), CYP 2A6 (Nicotine oxidation) etc. Thus identification of these genetic polymorphisms has now assumed a pivotal role in order to determine the nature of response to a drug and thus correctly determine the dose and choice of the therapeutic agent. This can be acccomplished by the process of genotyping that determines the differences in the genotype of different individuals.

Genotyping can be done by various methods such as restriction fragment length polymorphism identification (RFLPI) of genomic DNA, random amplified polymorphic detection (RAPD) of genomic DNA, amplified fragment length polymorphism detection (AFLPD), polymerase chain reaction (PCR), DNA sequencing, allele specific oligonucleotide (ASO) probes, and hybridization to DNA microarrays or beads.[4] This technique has not only revolutionized the world of science and cytochrome enzymes but has also revolutionized health care by enabling development of molecular models and appropriate dose adjustment to optimise drug therapy keeping in mind the variability in drug response amongst individuals based on CYP Polymorphisms. It has enabled

the understanding at a genetic and molecular level regarding the role of CYP in the pathogenesis of disease and development of these as potential drug targets. The field of drug development has been led into a new direction due to this recent advance.

Role of Cytochrome P450 in pathogenesis of disease

CYP 1, 2, 3 families play a very important role as illustrated above in terms of xenobiotic metabolism in mammals. Alterations in these enzymes may alter their physiological function of eicosanoid metabolism. If looked at individually with regards to their role in disease pathogenesis, primary congenital glaucoma is an autosomal recessive disorder caused by CYP1B1 mutations.[5] A recent study postulated that smoking via CYP mediated effects may be responsible for the enhanced HIV replication[6] and decreased antiretroviral drug efficacy thus providing newer dimensions for therapy in HIV infected smokers. A probable relationship has been established between CYP2D6 activity levels and behavior of individuals.[7] Anti CYP2D6 antibodies have also been postulated in Autoimmune Hepatitis type II and Cyp 2E1 is implicated in alcoholic liver disease and non-alcoholic steatohepatitis.[8] Overexpression of CYP 2J2 has been found in human hematologic malignant diseases.[9] Thirteen missense mutations in the CYP4V2 gene have been linked to Bietti crystalline corneoretinal dystrophy, an autosomal recessive disorder leading to progressive night blindness and constriction of the visual field.[10]

CYP 5A1 (TBXAS1) and CYP8A1 (PTGIS) also play an important role in eicosanoid metabolism in addition to the above gene families. Ghosal haemato-diaphyseal syndrome a rare autosomal recessive disorder has been found to be associated with four missense mutations in the TBXAS1 gene.[5] In a study,[11] an interesting fact noticed was one patient was found to have a splice-site mutation in intron 9, which manifested as a truncated protein bearing a deletion in the P450 haem-binding region which was also found in two of the siblings of the proband who were similarly hypertensive as the patient .

Amongst the enzymes involved in cholesterol synthesis and metabolism,CYP51A1 is the only P450 enzyme involved in cholesterol biosynthesis and is a common target of antifungal drugs (e.g. miconazole and ketoconazole), which inhibit CYP51 activity and formation of ergosterol. A homozygous frame shift mutation in the gene encoding CYP7A1 resulting in loss of enzyme activity is found to result in a metabolic disorder with elevated plasma cholesterol levels.[12] Increased risk for gallbladder cancer[13] and a decreased risk of proximal colon adenomas[14] has been associated with a CYP7A1 promoter polymorphism. Congenital bile acid defect type 3 can result from a homozygous mutation in the CYP7B1 gene of a neonate.[15]Mutations in the same gene have also been associated with hereditary spastic paraplegia (HSP) type 5A. Cerebrotendinous xanthomatosis is a rare autosomal recessive disorder with neurological dysfunction arising as a result of missense mutations in

International Journal of Pharmaceutical Sciences Review and Research
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286

the CYP 27A1 gene. Another CYP 46A1 is found to be expressed in the retina and polymorphisms have been found to be associated with age related macular degeneration. The above CYPs have also been used as new drug targets for cholesterol lowering agents in order to produce newer agents with improved efficacy.[16]

Shifting focus to the CYP's involved in steroid metabolism, severe defects in CYP17A1 result in 46 XX OR 46XY karyotype sexually infantile female while mild defects result in sexual ambiguity.[17] CYP 21A2 mutations are associated with congenital adrenal hyperplasia with degree of virilisation varying with levels of the enzyme.[18] CYP11B1 mutations result in 11-β-hydroxylase deficiency and defects in CYP11B2 result in rare forms of aldosterone deficiency. CYP 11A, CYP 21, CYP 17, CYP 19 have been extensively investigated as to being candidate genes in causation of Polycystic Ovary Syndrome.[19] Genes encoding CYP17A1 are also known to be targets of Endocrine Disrupting Chemicals (EDC'S).[20]

Amongst the enzymes involved in Vitamin D metabolism, a strong association between heterozygosity for CYP27B1 mutations and increased risk of multiple sclerosis has been postulated.[21] Also, deficiency of CYP24A1 causes severe infantile neonatal hypercalcaemia. [22]

The cytochromes have also been found to be associated with a variety of cancers and skin diseases. To mention a few, CYP1B1*3/*3 polymorphisms are found to be associated with increased risk of breast cancer and head and neck cancers in tobacco smokers. Overexpression of CYP2S1 and CYP2E1 has been found in psoriatic plaques.[23]

CONCLUSION

From the extensive list of physiological and pathological roles of CYP450 enzymes it is very clear that these biocatalysts demonstrate tremendous potential for further research and are sure to revolutionise drug development in the future. More emphasis to detect enzyme genetic polymorphisms and care regarding drug interactions is warranted to rationalise drug therapy.

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288