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PHENOTYPING – AN OVERVIEW

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ABSTRACT

The medicinal sciences have always aimed at an increased effort to increase the patient safety & reduce medical errors. These errors are mainly caused due to therapeutic failure and adverse drug reactions which arise as a result of incorrect dosing of the routinely prescribed drugs. The drugs are metabolized by drug metabolizing enzymes of which the Cytochrome P450 enzyme forms a major class. It is now being observed that with the descriptions of genetic polymorphism in the drug metabolizing enzymes, the field of pharmacogenetics may improve medical care through a reduction in adverse drug reactions oriented errors. The status of these drug metabolizing enzymes can be characterized using phenotyping studies which categorizes the population into poor, extensive or ultra-extensive metabolizers. Such a division of population based on their metabolic status will be of immense help to the medical authorities in deciding the drug dose. This paper reviews the field of Cytochrome P450 (CYP) genetics and explores factors that impact the utility of this information in clinical practice to avoid incidences occurring due to incorrect metabolism of the routinely prescribed drugs.

Key words: Phenotyping, Cytochrome P450, Drug metabolism

INTRODUCTION

The two areas that significantly represent patient's safety concern are – therapeutic failure & adverse drug events (ADEs). [1] Therapeutic failure refers to lack of efficacy due to lack of dosing where as ADEs include both compliance issues & medical dispensing errors. [2] On the other hand, adverse drug reactions (ADRs) are the complications that occur despite appropriate dispensing of the correct medication at the "intended dose".

The ADEs are usually caused when there is presence of excess drug in the body for a time longer than is necessary. The drug outcome is mostly based upon the genetic make-up of an individual which is likely to exhibit inter-individual difference in population owing to "genetic polymorphism". [3] Hence, the intended dose might not prove appropriate for every individual.

Studying polymorphic drug metabolism in a population helps to categorize individuals based on their ability to metabolize routinely prescribed drugs. Such studies are referred to as "phenotyping studies" and they are carried out by using an appropriate drug as a substrate for the respective enzymes and determining the drug-metabolite ratio. Based on this ratio the population is categorized into poor, extensive or ultra-extensive metabolizers.

These phenotyping studies would be an immense help to medical and therapeutic sciences to have prospective access to genetic information that might predict efficacy and/or toxicity of an individual for respective drugs.

Drug metabolism and Cytochrome P450 (CYP) Enzyme System:

Drugs are almost all xenobiotics. Drug metabolism is metabolism of drugs, their biochemical modification or degradation, usually through specialized enzymatic systems. **Drug metabolizing enzymes (DMEs)** often convert lipophilic chemical compounds into more readily excreted polar products. This drug metabolism can result in toxication or detoxication – the activation or deactivation of the chemical.

Drug metabolism is typically classified as Phase I and Phase II reactions. Phase I reactions are biotransformation reactions that alter the structure of the parent drug converting it to subsequent metabolites. Phase II reactions are usually known as conjugation reactions and they modify the drug covalently making it less toxic to body. Both Phase I & Phase II DMEs can impact a drug's activity.

Phase I DMEs include esterases, dehydrogenases, flavin monooxygenases and the Cytochrome P450 (CYPs). [4] Cytochrome P450 (CYP450) enzymes are essential for the production of cholesterol, steroids, prostacyclins, and thromboxane A2 and are located in the membranes of the smooth endoplasmic reticulum. They also are necessary for the detoxification of foreign chemicals and the metabolism of drugs. CYP450 enzymes are so named because they are bound to membranes within a cell (cyto) and contain a heme pigment (chrome and P) that absorbs light at a wavelength of 450 nm when exposed to carbon monoxide. There are more than 50 CYP450 enzymes, but the CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 enzymes metabolize 90 percent of drugs. [5, 6] out of these six enzymes, CYP2D6 and CYP3A4 is found to have maximum activity of 40% and 20% respectively [7] These enzymes are predominantly expressed in the liver, but they also occur in the small intestine (reducing drug bioavailability), lungs, placenta, and kidneys. [6]

Genetic Polymorphism and Cytochrome P450:

Genetic Polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles , in which the least common allele has a frequency of about 1% or greater. [7] One of the most extensively studied genetic polymorphism is the polymorphism observed in drug metabolizing enzymes. These changes in gene expression accompany drug-metabolizing enzyme gene polymorphism and cause alteration in enzymatic activity showing a gene-dosage effect. Genetic polymorphism has been found to categorize people into three different types of phenotypes based on their extent or

capability to metabolize a drug. Normal population is usually an **extensive metabolizer (EM)**; **poor metabolizers (PM)** show accumulation of the specific drug substance hinting at the deletion of or mutation in both alleles for phenotypic expression whereas **ultra extensive metabolizers (UEM)** exhibit an increased drug metabolism which is an autosomal dominant trait arising from gene amplification. [8]

A specific gene encodes each CYP450 enzyme. Every person inherits one genetic allele from each parent. Alleles are referred to as "wild type" or "variant," with wild type occurring most commonly in the general population. An "extensive" (i.e., normal) metabolizer has received two copies of wild-type alleles. Polymorphism occurs when a variant allele replaces one or both wild-type alleles. Variant alleles usually encode a CYP450 enzyme that has reduced or no activity. [9] Persons with two copies of variant alleles are "poor" metabolizers, whereas those with one wild-type and one variant allele have reduced enzyme activity. Finally, some persons inherit multiple copies of wild-type alleles, which results in excess enzyme activity. This phenotype is termed an "ultra extensive" metabolizer. [10]

CYP450 enzyme polymorphism is responsible for observed variations in drug response among patients of differing ethnic origins. [10, 11, 12] For example, 7 percent of white persons and 2 to 7 percent of black persons are poor metabolizers of drugs dependent on CYP2D6, which metabolizes many beta blockers, antidepressants, and opioids. [13, 14] One in five Asian persons is a poor metabolizer of drugs dependent on CYP2C19, which metabolizes phenytoin (Dilantin), phenobarbital, omeprazole (Prilosec), and other drugs. [15] Variance in drug response among persons of different ethnic origins also can be caused by genetic variations in other drug-metabolizing enzymes, drug transporters, and drug receptors. [16] The understanding of gene dosage effect related to the function changes of genetic polymorphisms of drug metabolizing enzymes, transporters and receptors not only provide potential novel insight into the effect of genetic polymorphisms on drug efficacy and toxicity but also point to the potential role of it in genotype directed tabloid drug therapy. Even though the additional larger and controlled studies are needed to justify changes of treatment strategies, the pharmacogenetics approach to individualize therapy in some patients is promising.

Phenotyping:

Phenotyping is one of the most common methods used to study genetic polymorphism. It requires intake of a probe drug; the metabolism of which is known to be solely dependent on one of the Cytochrome P450 (CYP) enzymes such as CYP2D6, CYP3A4 etc. The excretion of parent compound and/or metabolite in urine is used to calculate the metabolic ratio, which is the measure of individual' respective enzyme activity. [18, 19] Phenotyping studies can also be carried out by analyzing blood or saliva samples. [20, 21] This phenotyping study divides the population as per their genetic polymorphism into poor, extensive or ultra extensive metabolizers and thus can be used in deciding the drug dose for each individual. Defining the individual's phenotype, relative to a reference substrate, allows the drug metabolism phenotype for

other substrates of that enzyme to be predicted easily. [22]

Significance of phenotyping studies:

The clinical importance of various CYPs and their genetic polymorphisms has paved way for a new fields- "Pharmacogenetics" and "Pharmacogenomics". The traditional pharmacogenetic approach relies on studying sequence variations in candidate genes suspected of affecting drug response. On the other hand, pharmacogenomic studies encompass the sum of all genes, i.e., the genome. Thus, it can be said that pharmacogenomics has evolved from the academic science, pharmacogenetics, which focused primarily on genetic polymorphisms in DMEs. The "one drug fits all" approach of the Pharma industry could, with the fruits of pharmacogenomic research, evolve into an individualized approach to therapy where optimally effective drugs are matched to a patient's unique genetic profile. [23]

Standard drug doses may cause adverse effects related to elevated drug serum levels if a person is a poor metabolizer or has a CYP450 enzyme inhibitor added to therapy. [24, 25] Adverse effects are more likely to occur if a drug has a narrow safety range or is dependent on only one enzyme for metabolism. This can be best explained by the following illustrations.

Illustration 1: A 35-year-old white woman with panic disorder was treated with paroxetine (Paxil). She developed unrelated hypertension, for which the physician prescribed 50 mg daily of extended-release metoprolol (Toprol XL). The patient became symptomatically orthostatic after a few days and presented to the emergency department. In this example, metoprolol, which is metabolized solely by CYP2D6, was present in higher serum levels in the patient because of the use of paroxetine.

Peak serum levels of simvastatin (Zocor), which is metabolized solely by CYP3A4, also can increase by many times in patients who are poor metabolizers or with the addition of a potent inhibitor, increasing the risk of myopathy and rhabdomyolysis at usual doses. [26]

Illustration 2: The linkage between the HER2/neu oncogene and the efficacy of Herceptin® for the treatment of advanced breast cancer is another example where pharmacogenomics has proven successful. Profiling of subjects with breast tumours expressing HER2/neu (class 2-3 HER2/neu immunohistochemitsry staining) was utilised to compare clinical efficacy in late stage drug development with those individuals most likely to respond to therapy. [27] The linkage between HER2/neu levels assessed by a diagnostic test and selection of therapy with Herceptin® is one of the first pharmacogenomic applications approved by the FDA. [28] This is also an excellent example demonstrating that each patient (and his disease) is highly variable, requiring treatment based on the expression of tumour genes. Only 25 - 30% of all breast cancers overexpress HER2/neu, effectively segmenting the market for Herceptin®. However, physician and patient confidence in selecting this therapy based on a prospective pharmacogenomic approach will actually result in better disease management and compliance.

Ultimately, this approach will mean that positioning and differentiation of drug treatment will allow drugs that would otherwise not be approved to be used in a

select population of patients, with premium pricing and better acceptance. [28]

Methodologies used in phenotyping:

Probe substrates (or probe drugs) are compounds that are predominately or exclusively metabolized in vitro by an individual CYP enzyme. [29] The metabolism of the candidate probe is generally characterized through the use of preparations containing individually expressed human CYP enzymes or preparations of human liver microsomes. [30] Drugs that are selectively metabolized and that can be safely administered to humans may be used as in vivo probe drugs for the purposes of phenotyping i.e., estimating CYP enzyme activity.

1) Single drug probes- The phenotyping procedure typically involves the administration of the probe drug and the collection of blood and/or urine in order to determine some measure of the enzyme's functional activity. Typically, as small a dose of the probe drug as possible is administered so as to avoid or minimize undesirable clinical effects. An index of enzyme activity, also referred to as a phenotypic trait measure, is chosen to reflect the catalytic activity of a single pathway of metabolism. The intrinsic clearance of a probe or of the metabolite(s) produced, termed formation clearance, is the most appropriate measure of enzyme activity. [31]

2) Cocktail method- Administering multiple probe compounds concomitantly, termed the "cocktail" strategy, is a useful method in the assessment of drug-metabolizing enzyme activities because it allows for the in vivo assessment of multiple pathways of drug metabolism in a single experiment. [32] The utility of the cocktail strategy, first demonstrated by Schellens and Breimer in multiple investigations, offers several potential advantages in that it reduces participation time for the study subjects and increases efficiency for the investigators by decreasing time and expense. [32] More importantly, this approach minimizes intra-individual variability since the evaluation will occur on one day rather than separate days.
Phenotypic data (i.e., enzyme activity) can be obtained simultaneously on multiple pathways while genetic material obtained can be examined for known or novel SNPs in the drug-metabolizing enzyme genes. Thus, the cocktail strategy appears to be an invaluable method to investigate differential modulation of CYP activity. [33]

Current status of phenotyping:

Around the world- Several phenotyping studies have been conducted around the globe to determine the phenotype status of an individual. These studies have not only been conducted for different population in various parts of the world but also with respect to different CYP enzymes. Based on these studies different percentages of PM, EM and UEM have been obtained for different enzymes based on the inter-ethnic differences in the population. For example, CYP2C19 the prevalence of PMs has been reported to be 2–5% in Caucasians [34, 35] 4–8

% in Africans [36] and 11–23 % in Orientals. [35]

In Maharashtra: As far as the state of Maharashtra goes, only one such phenotyping study has been conducted in the Mumbai. This study was conducted to study the genetic polymorphism in CYP2C19 enzyme in the Gujrathi and Marwadi population of the Mumbai region. The probe drug used to estimate the enzyme activity was omeprazole (20 mg) whose drug metabolite is 5-hydroxyomeprazole. The DM ratio analyzed on HPLC (High Performance Liquid Chromatography) led to the generation of the phenotyping data. It was seen that 10.36% of this population were poor metabolizers (PM) whereas 89.63% were extensive metabolizers (EM). [36]

CONCLUSION

Genetic polymorphism appears to be a significant source of variability observed in the response to drugs. This variability means that information pertaining to interethnic and inter-individual genetic differences can be used to facilitate rational drug discovery and development and to avoid or minimize the incidence of adverse events in clinical trials. Thus, one could generate criteria for selecting patients most likely to benefit from a drug without incurring unnecessary risk.

In this review we have seen the clinical application of phenotyping data as they apply to the clinical laboratory as well as to the clinical practitioner. Phenotyping may be indicated in each instance when the therapeutic of choice is a substrate for a polymorphic enzyme. Alternatively, genotyping is indicated when individuals demonstrate suboptimal response to drugs that are substrates for polymorphic enzymes. The advantage of combining phenotyping with therapeutic drug monitoring is that genotyping can predict the PM or UEM drug metabolism phenotypes, and this information can be used in prior dose adjustment or selection of an alternative therapeutic that is not a substrate for the polymorphic enzyme. The cost/healthcare effectiveness of these paradigms has not been extensively studied. Although there would be considerable cost associated with screening all individuals before dosing with these drugs, this cost may be offset by a reduction in costs associated with toxic episodes or therapeutic failure and subsequent intervention.

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