CHAPTER -1

INTRODUCTION
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Introduction and objectives

The practice of drug development includes an understanding of the processes involved in drug handling and action. This includes knowledge of the absorption, distribution, metabolism and elimination of the drug by the body. This knowledge leads to the selection of a range of safe and effective doses for the population intended to be treated. All drugs exhibit significant inter-subject variability in pharmacokinetics and pharmacologic response. Such differences vary considerably among individual drugs and depend on a variety of factors, which are often separated into extrinsic and intrinsic factors.

One of the most important factors affecting drug metabolism is the existence of genetic polymorphism of drug metabolizing enzymes that leads to altered drug metabolizing enzymes. The main cause of the genetic polymorphism is the differences in a single nucleotide in the genetic code, which in turn influence the enzyme expression or protein structure. Genetic variations refer not only to gene alteration, but also to gene regulation, which results in the expression of different amounts of proteins. All of these changes affect drug metabolism.

A number of polymorphisms in drug metabolizing enzymes have been discovered in the last few decades. Polymorphism of Cytochrome P450 enzymes, the most common drug metabolizing enzyme significantly affects the pharmacokinetics of about 50% of the drugs in clinical use. The consequences of the polymorphism at ordinary drug doses can be either adverse drug reactions or no drug response. Other potentially important determinant of drug responsiveness is ethnicity. The influence of ethnic factors on drug disposition and pharmacologic response has been extensively reviewed. Same enzyme behaves differently in various ethnic populations and this
functional difference is due to genetic variation in these drug metabolizing enzymes. Hence it is a key consideration to identify the genetic polymorphism in the different regions. To address this issue, the ICH (International Conference on Harmonization) and the United States Food and Drug Administration recommended a framework for evaluating the impact of ethnic factors on a drug's safety and efficacy at a particular dosage and dose regimen, involving regulatory and development strategies that will enable appropriate evaluation of ethnic factors.

Most of the current literature is concerned with genetic polymorphism in Caucasians, African-Americans whereas the information on genetic polymorphism in Asian population is scanty. Polymorphism of the most commonly involved drug metabolizing enzymes CYP2D6, CYP3A4 and CYP2C19 is very well established for northern Indian population. However, little information on polymorphism of phase 1 enzyme CYP2B6 is available. It may be due to very less proportion of CYP2B6 enzyme compared to other enzymes- CYP3A4, CYP2D6, CYP3A42C9, CYP2C8, and CYP2C19. Although, the proportion of this enzyme is less but number of clinically important drugs involved in the treatment of cancer and depression is metabolized by this enzyme. In the present study genetic polymorphism of CYP2B6 enzyme is evaluated. CYP2C9 is another clinically important drug metabolizing enzymes which is also known to be polymorphic in nature and polymorphism of this enzyme has not been earlier reported for the NCR Delhi population hence selected in the present study. Apart from these two CYP enzymes, the polymorphism of a phase 2 enzyme N- acetyltransferase 2 (NAT2) was evaluated in the present study as the prevalence of poor acetylators is reported to be high in Indian population as compared to other phase 2 enzymes. Moreover NAT2 and CYP2C9 have not been evaluated with CYP2B6 in a cocktail method to evaluate the phenotypes.

Phenotyping of individual enzyme is a time consuming and is not a cost effective technique. The cocktail approach made it possible to assess multiple enzymes at the same time. Phenotyping using cocktail approach substantially lower clinical cost and
time. The effect of intra individual variability is also minimized. The limitation to the cocktail approach is the presence of any in vivo interaction between the individual probes present in the cocktail and the availability of appropriate analytical method to estimate the drug levels in the presence of cocktail drugs and their metabolites. In the present study all these concerns have been addressed.

In this study, a cocktail of three drugs is selected to evaluate the activity of CYP2B6, CYP2C9 and NAT2 simultaneously. The activity of CYP2C9, NAT2 and CYP2B6 is assessed by (losartan/losartan carboxylic acid), (dapsone/monoacetyldapsone), and (bupropion/hydroxybupropion) ratio respectively. Although phenotyping is an excellent tool to phenotype population as rapid and poor metabolizers using probe drugs, but this technique is time consuming and tedious hence the alternative technique genotyping methodology is planned with the identification of known SNPs (single nucleotide polymorphism) to have a better correlation of phenotyping and genotyping techniques. Phenotype-genotype if correlated, in this population with the identification of known SNPs would be tool to further phenotype any individual either by phenotyping or by genotyping technique. However the genotype results in case is not in consensus with phenotyping results, would directed that genotyping alone would not be sufficient and complete analysis for the phenotyping of individuals for these drug metabolizing enzymes is required. Overall the rationale for conducting the present study can be summarized as follows:

1. The cocktail approach can simultaneously assess multiple enzymes, for example, CYP2B6, CYP2C9 and NAT2, which reduce the intra-individual variability.

2. The study is useful in therapeutics, i.e. to decide the dose for a patient.

3. Identification of the phenotypes can be a tool to prevent drug toxicity in case the prevalence of poor metabolizers for the category of drugs metabolized by these enzymes is significantly high.

4. The cut off calculated in phenotyping procedure may be further used to have baseline information of drug metabolizing status of CYP2C9, CYP2B6, and NAT2.
5. Genotyping and phenotyping technique if correlates, would be a tool to further phenotype any individual by genotyping technique which is relatively safer (no involvement of drug dosing required) and time saving technique.

6. CYP2C9, CYP2B6 and NAT2 enzymes selected in the study have never been evaluated simultaneously using cocktail drug approach.

7. The selection of losartan, bupropion and dapsone was done on the basis of its easy availability, safe usage and earlier recommendation to use as a probe drugs to evaluate the activity of study enzymes.

8. The LC-MS/MS method developed and validated in the study, to quantify the three study drugs and their metabolite after the simultaneous administration was never established earlier.

Briefly, the objectives of the study are:

1. To develop and validate a cocktail that can be helpful to determine the activity of the phase 1 drug metabolizing enzymes, CYP2C9 and CYP2B6, and phase 2 enzyme NAT2 in a single trial using commercially available drugs losartan, dapsone and bupropion. The validated cocktail will be useful in promoting individualized drug therapy for diseases in which these drugs are used.

2. To determine the prevalence of slow, intermediate and rapid drug metabolizers for the category of drugs metabolized by cytochrome P450 enzymes CYP2B6 and CYP2C9 and the phase 2 drug metabolizing enzyme, N-acetyltransferase in the NCR Delhi population, for which the data is not available.

3. The present study is also extended to genotyping of the above enzyme/s to have a relationship between genotyping and phenotyping of these enzymes.