CHAPTER 8

SUMMARY

AND

CONCLUSION
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The current ‘one-medicine- fits all’ approach cannot satisfactorily treat all patients efficiently and to understand why individuals respond differently is the most challenging research area in drug therapy. With a better understanding of genetic variants of drug metabolizing enzymes, it is hoped that maximum drug response can be achieved in drug therapy by compensating for individual variability. Genotyping or phenotyping of patients for drug metabolizing enzymes in clinical trials is a tool to evaluate these genetic variants or polymorphs.

The aim of the present study was to evaluate the intrinsic activity of CYP2C9, CYP2B6 and NAT2 in a single trial using cocktail approach in the NCR Delhi population that caters to Clinical Pharmacology Unit of Ranbaxy Laboratories Limited. The proposed cocktail was comprised of sub-therapeutic doses of three drugs, dapsone, losartan and bupropion. The three drugs cocktail was well tolerated by the volunteers with no adverse events observed. This three drug cocktail is probably the first time given in combination for the simultaneous assessment of the above three enzymes activity. The activity of CYP2C9, CYP2B6 and NAT2 was assessed as ratio of losartan and losartan carboxylic acid; bupropion and hydroxybupropion; and dapsone and monoacetyl-dapsone. It has been known that there could be an analytical, pharmacokinetic and clinical interference when two or more drugs are administered as a cocktail, hence it is mandatory to evaluate analytical, pharmacokinetic and other clinical interferences in cocktail studies. In the present study, all of these concerns were addressed. Number of analytical methods are available to determine these probe drugs and their metabolite concentration, however none of the method was available to quantify these probe drugs and their metabolite in a plasma sample of human volunteers, administered a
cocktail of losartan, bupropion and dapsone. The LC-MS/MS methodology described in chapter 4 was precise and accurate which was verified with quality control samples and reference standards. There were no analytical interferences were observed as no interfering peak was observed at the retention time of drugs and metabolites. Moreover all the validation parameters sensitivity, specificity, precision, accuracy and storage stability were well within the acceptable limits as defined by FDA validation criteria. The present methodology has an advantage of low retention time. Low retention time will reduce the time of analysis. Pharmacokinetic parameters $T_{\text{max}}$ and $C_{\text{max}}$ calculated from clinical samples were comparable to the earlier reported values. The LC-MS/MS methodology developed was applied for the phenotyping of above three enzymes in NCR Delhi population.

Genetic polymorphism of $CYP2C9$ in NCR Delhi population was evaluated and described in chapter 5. It is probably the first attempt to establish the phenotype-genotype correlation of $CYP2C9$ polymorphism in NCR Delhi. Losartan used in phenotyping of $CYP2C9$ enzyme found to be safe, suitable and tolerable drug with no adverse event observed during the clinical trial. The cut off calculated to distinguish poor metabolizers from rapid metabolizers is 0.73. The individuals having log ratio of losartan/losartan carboxylic acid more than 0.73 were categorized as poor metabolizers. Based on the cutoff, 14.28% of study population was poor metabolizers. The fact that 14.28% of the study population belonging to NCR Delhi is poor metabolizer for the categories of drugs metabolized by $CYP2C9$ make it necessary to identify the status of individuals for clinical management of drug dose for any long duration therapy or in clinical trials to prevent adverse reactions of the drug. The volunteers were genotyped for the identification of single nucleotide polymorphism (430C>T); (1075A>C) for the identification of $CYP2C9^*2$ and $CYP2C9^*3$ variants. Genotyping analysis revealed that all the poor metabolizers had $CYP2C9^*2$ mutation either in homozygous or heterozygous form. $CYP2C9^*3$ variant was not observed in the current population. Phenotype-genotype correlation revealed 100% correlation with the rapid and poor metabolizers and concludes that genotyping or phenotyping may be used to evaluate the status of
drug metabolizing capacity of CYP2C9 as a primary screening procedure before enrolling subjects for clinical trials or in clinical practice.

Bupropion as a probe drug used in phenotyping of CYP2B6 enzyme was found to be safe and well tolerated in the present study. This study described in chapter 6 is the first attempt to identify poor and rapid metabolizers of the drugs metabolized by CYP2B6 in north Indian population residing in the national capital. The Individuals having log ratio of hydroxybupropion/bupropion > 0.5 were categorized as rapid metabolizers. 20.56% of population were identified as poor metabolizers in NCR Delhi population which cannot be ignored as this enzyme is involved in the metabolism of drugs commonly used for the treatment of cancer, HIV infection and as an antidepressant which are long term treatment and could resulted into toxicity in poor metabolizers. The difference in the mean ratio (bupropion/hydroxybupropion) in poor and rapid metabolizers was highly significant indicating toxicity of bupropion in poor metabolizers. The antimode or cutoff defined in this study can be used as a tool for evaluating the status of CYP2B6 activity using bupropion as a probe drug. In this study, we could not evaluate the correlation of phenotype with genotype, which would be advantageous to understand the genetic background of the difference in poor and rapid metabolizers.

In chapter 7, the prevalence of poor and fast acetylators in NCR Delhi population was determined by the assessment of NAT2 enzymatic activity using dapsone as a probe drug. Dapsone is proven to be safe and tolerable with no adverse event reported. In the present population 57% of the individuals were found to be poor acetylators in the study population. The difference in the mean ratio (dapsone/monoacetyldapsone) in poor and rapid metabolizers was highly significant indicating toxicity of dapsone in poor metabolizers. In the present study, \textit{NAT2*5B and NAT2*6A} was present in majority of the subjects as compared to \textit{NAT2*5C, NAT2*12A, NAT2* 6C, NAT2*7B}. Studies have shown that 5B/6A genotype are more susceptible to drug induced toxicity, hence the evaluations of acetylator status is highly important to decide the dose of drugs in this
population. This study also demonstrates a good genotype-phenotype correlation hence either of these techniques can be used to evaluate acetylator status in NCR population for clinical trials or for deciding drug dose regimen.

Conclusion

I) The study is an effort to identify the polymorphism of CYP2C9, CYP2B6 and NAT2 enzymes of NCR Delhi population. This would help to provide appropriate dosage of drugs to individuals based on their genotypes and to identify drug induced toxicity. The present cocktail may be applied in drug development to study the effect (inhibition or induction) of a new chemical entity in vivo on individual CYP enzymes. This cocktail approach can be used to evaluate the polymorphic enzymes activity in a single trial in different populations.

II) The LC-MS/MS method developed and validated for the estimation of three drugs losartan, bupropion and dapsone; their metabolites losartan carboxylic acid, hydroxybupropion and monoacetyldapsone after the simultaneous administration of these three drugs was a never developed earlier. This method was applied for the phenotyping of CYP2C9, CYP2B6 and NAT2 enzyme. This method has an advantage of low retention time that will reduce the time of analysis.

III) As the genotype-phenotype correlation was established in the present study, an individual’s status as rapid or poor metabolizer can be ascertained by genotyping or phenotyping technique alone for the evaluation of CYP2C9, CYP2B6 and NAT2 enzymes. Genotyping being a relatively easier technique as compared to elaborating process of phenotyping technique may be the choice for future categorization of individuals as rapid or poor metabolizers.
IV) The antimode or the cutoff value defined in the present study would be a tool for evaluating the status of CYP2B6, CYP2C9 and NAT2 enzyme activity using bupropion, losartan and dapsone as a probe drug. The baseline information about the polymorphism of this enzyme would be clinically useful for the individualize drug treatment for all those drugs that are substrates of the above enzymes.