CHAPTER-1

INTRODUCTION

1.1. DRUG-DRUG INTERACTIONS

The change in clinical response when the patient is under medication on a drug due to administration of another drug can be called as Drug-Drug Interaction (DDI). Without affecting the drug kinetics, when a change in pharmacological response occurs by agonism or antagonism these DDIs are known as pharmacodynamic interactions. Alterations in drug ADME due to enzyme/transporters up regulation or down regulation, these DDIs are considered as pharmacokinetic interactions [1]. DDIs are responsible for approximately 20-30% of the ADRs in general population and account for about 10% of the cases under emergency department and they also contribute 3-5% of the medication errors in hospitalized patients [2,3].

Drug pharmacokinetics can be significantly altered by both transporters and enzyme mediated DDIs and hence the therapeutic efficacy or toxicity of drugs can be potentially affected [4]. DDIs based on metabolism are specifically due to induction and/or inhibition of cytochrome P450 has been considered to be the most dangerous one [5]. The clinical consequences of CYP induction or inhibition depends mainly if the effected drug has a narrow therapeutic index and is significant. [6]. Thus, when the parent drug’s metabolic pathway is affected, it alters route of elimination, induction and inhibition leading to toxic effects or no pharmacological activity. The other way, if the parent compound is a prodrug, enzyme induction may lead to potential toxicity where as inhibition of its metabolic conversion may cause a reduction in therapeutic efficacy. When reactive metabolites are generated, induction may be dangerous as they can cause serious idiosyncratic reactions frequently [7]. A recent systematic review revealed that, based on the in vitro tests performed at the time of drug development, majority of the new chemical substances were perpetrators of metabolic interactions. It has been found that 45% of the new chemical entities are the effectors of clinically significant metabolic DDIs [8]. By the FDA regulatory guidance, more complex and refined predictive models have been proposed and endorsed subsequently. Recently, it has been pointed out that they do not enhance
predictive capacity. The underlying mechanism that alters the drug disposition in liver disease is unknown and hence it is difficult to predict the effect of liver dysfunction on metabolism based DDIs [9, 10,11].

1.2. DRUG METABOLIZING ENZYMES

Drug metabolizing enzymes can be categorized into two groups.

(1) Oxidative drug metabolizing enzymes
   i. Cytochrome P 450 (CYP 450)
   ii. Flavin monooxygenases (FMOs)

(2) Conjugative drug metabolizing enzymes
   i. UDP-glycosyl transferases (UGTs)
   ii. Glutathione transferases (GSTs)
   iii. Sulfonyl transferases (SULTs)
   iv. N-acetyl transferases (NATs) [12,13]

1.3. CYTOCHROME P 450 ENZYME SYSTEM

In the metabolism of a wide variety of both exogenous and endogenous compounds CYP P450 enzymes are involved[12]. In the year 1955, they were first discovered in rat liver microsomes. In the presence of carbon monoxide, they are characterized by an intense absorption band at 450nm [13]. On the smooth endoplasmic reticulum, the cytochrome P450 mixed function mono oxygenases are located throughout the body. Small intestine and liver are the major sites which were observed with highest concentration [14]. Oxidative (Phase -1) metabolism is most common for many compounds. Lipophilic drugs can be biotransformed to more polar compounds that are eliminated by the kidneys. In some instances the metabolites can be toxic, teratogenic and even carcinogenic. In humans, about 12 cytochrome P450 gene families have been known. In majority of the drug biotransformation, three families are mainly involved that includes cytochrome P450 1, 2 and 3 (CYP-1, CYP-2 and CYP-3). Based on the similarities of amino acid sequence, these enzymes are divided into families. Further, each family can be divided into subfamilies. These are designated by capital letters followed by the family designation (e.g. CYP 3A).
Subsequently, individual enzymes are indicated by Arabic numbers (e.g. CYP 3A4). A single liver cell can have a variety of cytochrome p-450 enzymes. In humans, the most abundant cytochrome enzymes are the members of CYP3A sub family that includes 70% of the enzymes are in the gut and the remaining 30% were in the liver. In the adult liver, the major form of cytochrome P 450 is the CYP3A4 which metabolise the larger proportion of drugs. CYP 3A4 and CYP 3A3 cannot be distinguished from each other because they have more similarities in metabolizing the substrates. In stomach, the major enzyme is the CYP 3A5 and is present in only 20-30% of the Caucasians. The deficiency in CYP 3A5 does not cause any problem because all the major functions can be carried out CYP 3A4. CYP450 enzymes are the most common causes of altered drug biotransformation reactions i.e., induction and inhibition [15,16,17].

**Induction**

Increase in synthesis of enzyme that is associated with exposure to drugs is called as Induction. When a drug modifies the metabolism of co-administrated drugs, the induction can occur. This may occur through the same enzyme pathway or via an alternative pathway. For a given cytochrome P450 family, specific inducers are usually present. In addition to other agents, sometimes a drug can induce its own biotransformation. Within the first two days of treatment, effects of induction can be seen. For the synthesis of new enzyme, it usually takes more than one week.

**Inhibition**

The most common mechanism of inhibition is the competitive inhibition that occurs when 2 or more drugs compete for the same enzyme. The clinical significance of an inhibition interaction depends primarily on the drug’s relative concentration and also on various patient specific factors. Drugs can bind with haem–binding site reversibly or irreversibly. This inhibits the binding of other drugs. By the cytochrome P-450 system, some drugs undergo metabolic activation. Stable complexes with cytochrome p-450 can be generated by the metabolites so that the cytochrome is held in an inactive state. Since it is relatively long in duration, there can be great clinical significance to this interaction. There is a chance of toxicity, when the interaction involves narrow therapeutic drugs. [18, 19].
1.3.1. Cytochrome P450 Enzyme System in Hepatic Dysfunction

The mechanism and the pathogenic significance remain unclear. Various studies revealed the role of CYP2E1 in the pathogenesis of non-alcoholic steatohepatitis and alcoholic liver disease. Increased CYP2E1 activity leads to lipid peroxidation and the generation of reactive oxygen species with secondary damage to mitochondria and cellular membranes. It has also been postulated that CYP2E1 has a role as a cofactor for hepatocellular carcinoma due to its ability to activate carcinogens. Particularly for the drugs mediated by CYPs, drug metabolism can be impaired in patients with hepatic disease.

The activity and content of CYP1A, 2C19 and 3A effect in liver disease where as CYP2C9, 2D6 and 2E1 are less affected. Based on the etiology of liver disease, the pattern of CYPs isoenzymes alternations also varies. To assess the hepatic functional reserve, the usefulness of measuring CYP activity remains uncertain [20].

Effect of Hepatic Dysfunction on Enzyme Inhibition

In hepatic dysfunction, five types of factors can affect the extent of inhibitory DDIs.

1. Decreased enzyme content.
2. Hepatic effecting ratio on drug with inhibited metabolism.
3. Decreased liver uptake of the inhibitor.
4. Nature of the inhibitory interaction (reversible or irreversible).
5. Plasma protein binding of the drug with inhibited biotransformation.

1. Decreased enzyme content

Decreased enzyme(CYP1A2 and CYP3A4) content results in reduced inhibitory effect [21, 22].

2. Hepatic effecting ratio of the drug with inhibited metabolism

The clearance of drugs with a high extraction ratio can be determined by liver perfusion and should be unaffected by a reduction in intrinsic clearance caused by enzyme inhibition [23].
3. Decreased liver uptake of the inhibitor

For various basic drugs which are structurally unrelated, decreased drug uptake in cirrhosis of liver has been seen by in vitro models. [24].

4. Nature of the inhibitory interaction (reversible or irreversible)

Liver dysfunction may differentially affect the accumulation kinetics in the liver cell of reversible and irreversible inhibitors.

5. Plasma protein binding of the drug with inhibited biotransformation

The reduction in plasma protein concentration associated with hepatic dysfunction enhances the magnitude of drug interactions consequent to plasma protein-binding displacement. The consequences of enzyme inhibitions are masked by the displacement, the effect on the total plasma clearance of the victim drug by any perpetrator causing plasma protein binding displacement and metabolic inhibition may reduce with the increase in liver dysfunction. [25, 26].

1.3.2. Cytochrome P450 Enzyme System in Diabetes Mellitus

The changes in the cytochrome P450 (CYP) and FMO expression and function in the liver and intestine can be produced by Insulin dependent diabetes mellitus (IDDM). The metabolism in IDDM medicated by CYP and FMO was investigated by streptozotocin (STZ) induced experimental diabetes model. Due to the hormonal and metabolic changes associated with diabetic state usually the alterations of cytochrome P450 enzyme system takes place in both the organs.

In the regulation of these enzymes insulin plays a crucial role either directly or indirectly via insulin signaling pathway because, majority of the alterations are either completely or partially restored following treatment with insulin. In experimental diabetes, CYP2C11 and CYP2C13 are down regulated. In diabetes, alterations of CYP2C22 and CYP2C23 are less reported. In diabetes, the effect of altered CYP mediated metabolism on newly developed drugs or clinically used drugs is less investigated. Screening of drug safety plays a significant role in the process of drug research and development [27].
1.3.3. Cytochrome P450 Expression and Activity in Liver with Diabetes Mellitus

In drug biotransformation, the interindividual variations can be caused due to the variability in plasma drug concentration among the patients receiving the same dosage and this result majorly from variability in the expression of cytochrome P450. Approximately 96% of xenobiotic metabolism are due CYP 450 (1, 2 and 3 families). The 3A sub family comprises of 3A4, 3A5, 3A7 and 3A43 which have different expression patterns and similar substrates specificities. For about 55% of the marketed drugs and as well as many endogenous substrates the cytochrome P450 3A sub family contributes to the biotransformation. In the liver and intestine, CYP 450 3A4 is the most abundant one. When compared to 3A4, 3A5 has less significance because it is less expressed in liver and intestine. The human cytochrome P450 genes are highly diversified and various alleles have been present and in this CYP3A4 allele demonstrate that variants lead to change in enzyme activity.

Between the CYP3A4*1B allele and the pharmacokinetics variability of some P450 3A4 substrates inconsistent results have been observed. With an allele frequency of about 90% in Caucasians the CYP3A5*3 variant is the most common defective CYP3A5 allele. In both in vitro and in vivo clearance of several P450 3A substrates, a marked reduction can be observed with this variant. The transcriptional regulation of CYP 450 3A through the nuclear receptors Pregane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) in CYP 3A gene expression interindividual variation can be seen and thus, the drugs which are the ligands of CAR and PXR transcriptionally modulate the expression of P450 3A. The expression of CYP450 not only modulated by different chemicals, but also by pathophysiological conditions like diabetes. Due to various factors like age, gender, degree of diabetes control and disease duration, the literature available on the effect of diabetes on CYP 450 activity in humans is very contradictory and more over, the molecular mechanisms that are responsible for these alterations in drug biotransformation have not been completely characterized yet [28].
1.4. DRUG TRANSPORTERS

A vast number of membrane transport proteins have been found during the last two decades. In the absorption, distribution and elimination of drugs, these transporters are the significant determinants.

Drug transport proteins were categorized into two classes

1. Solute carriers (SLC) transporters.

2. ATP –Binding Cassette (ABC) transporters.

For ABC transporter gene, around seven sub families were identified, encoding for 49 different proteins. In particular, ABC transporters belonging to the ABCB, ABCC and ABCG sub families, a number of them have specificities of drugs, in the transport of a wide range of substrates through SLC and ABC transporters. [29-30].

Transporters Role in Pharmacokinetics

Drug size, lipophilicity and charge are some of the physicochemical properties of the drug that generally affect the extent of drug movement through these membranes. For example organic anion transporting polypeptide 1B1 (OATP 1B1) an uptake transporter rip out its drug substrates into hepatocytes from the portal blood [31, 32,33].

In the efflux of the metabolite form the hepatocyte, other drug transporters like multidrug resistance protein-1 (P- glycoprotein) may be important after metabolism.

Organic Anion Transporting Polypeptides (OATPS)

OATPs transporters regulate the various endogenous and clinically significant drugs. The human OATP family comprises of 11 members that include OATP 1A2, 1B1, 1B3, 1C1, 2A1, 2B1, 3A1, 4A1, 4C1, 5A1 and 6A1. In drug pharmacokinetics, the roles of OATP 1B1, 1A2, 1B2 and 2B1 are best characterized. These transporters mediate the influx of their substrates into the hepatocytes from the blood and facilitate elimination.

All OATPs share an identical transmembrane domain organization with a large fifth extra cellular loop and 12 predicted transmembrane domain as per a computer based
hydropathy analysis. The transport of all OATPs occurs through a central, positively changed pore in a so called rocker-switch type of mechanism.

**Types of OATP Transporters**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Type of transporter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OATP 1B1</td>
<td>OATP 1B1 was expressed in human hepatocytes of sinusoidal membrane. In the enterocytes of small intestine, SLCO 1B1 mRNA has been detected. Both unconjugated and conjugated bilirubin are transported by OATP 1B1. Examples of in vitro OATP 1B1 drug substrates are HMG-COA reductase inhibitors or statins.</td>
</tr>
<tr>
<td>2</td>
<td>OATP1A2</td>
<td>It is found in brain, liver, intestine and kidneys. Bile acids, steroid hormones and thyroid hormones are the endogenous substrates of OATP 1A2.</td>
</tr>
<tr>
<td>3</td>
<td>OATP1B3</td>
<td>Based on the sequence homology to the OATP1B1, OATP1B3 was cloned and is seen on sinusoidal membrane of human hepatocytes. Bilirubin, conjugated steroids, bile acids, thyroid hormones and eicosanoids are the endogenous substrates of OATP1B3.</td>
</tr>
<tr>
<td>4</td>
<td>OATP2B1</td>
<td>It is expressed in the liver heart and intestine. Estrone -3-Sulfate, prostaglandin E2 and de hydroepiandrosterone-3-sulfate are the endogenous substrates of OATP2B1.</td>
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</tbody>
</table>

**ABC Drug Transporters**

ABC transporters play a significant role in the absorption, distribution and removal of drugs and are expressed in epithelia of liver, intestine and kidney. ABC transporters are categorized involved into ABCB, ABCC and ABCG families. The molecular weight can be 150-200 kilo Daltons and made up of two transmembrane domains, each constituting of six transmembrane helices and two cytoplasmic ATP- binding domains. To make primary active drug efflux they bind and hydrolyze ATP that is
directly linked to their ATPase activity of two cytoplasmic ATP-binding domains [34].

P-gp has broader substrate specificity with ranging from small molecules of 350 Daltons up to polypeptides of 4000 Daltons. A high number of P-gp substrates belong to bulky, polyvalent, organic-cations and is articulated in apical membranes of intestine, kidney epithelia and liver. It decreases systemic drug exposure by reducing the oral absorption and enhancing biliary and urinary excretion [35,36].

ABCC sub family consists of nine multi drug resistance protein (MRP) members have been found, among them ABCC2, ABCC3 and ABCC4 are important drug transporters. Majority of organic anionic compounds can be mediated by MRPS in liver, intestine and kidneys [37].

1.4.1. Transporters for Hepatic Drug Elimination

With high protein binding from the circulation, the liver has the capability to extract the drugs efficiently. The hepatic uptake of drugs follow phase-I and phase-II metabolisms. In drug elimination process, it has been recognized that influx and efflux transporters are censious determinants. OATP1B1, OATP1B3, OATP2B1, OAT2 AND OCT1 are the drug influx transporters expressed at the sinusoidal membrane.

For majority of clinically relevant drugs like statin, macrolide antibiotics, sartan, glitazones and angiotensin converting enzyme, OATP1B1 is recognized to be most significant uptake transporter. Clinically relevant drug-drug interaction was found between cyclosporine-A and OATP 1B1 mediated transport have been described [38]. Similar substrate specificity has been observed for the homolog OATP1B3 and it is only expressed in the liver cells encircling the central vein. The activity of these transporters is often the rate limiting step in hepatobiliary drug elimination and the interindividual variation in drug exposure and disposition depends on inhibition and genetic variability drug transporter. With high statin blood concentrations, these genotypes are known to be associated [39].

The influx of type I organic cations into liver cells was mediated by OCT1 and also facilitate the efflux of cationic drugs back in to the blood as a bidirectional
electrogenic uniporter. This all depends on the electrochemical gradient. Among the most commonly prescribed drugs for the treatment of type-II diabetes, metformin is the most clinically important substrate and its antidiabetic action is dependent on uptake into liver cells [40,41,42].

MATE 1 and MDR 1/P-gp mediates the excretion of type I and II cationic drugs respectively across the canalicular membrane. For MATE 1, metformin is a good substrate and by active tubular secretion, the drug is mainly excreted into urine. For the canalicular efflux of conjugated and unconjugated anionic drugs, MRP2 and BCRP are primarily responsible. [43,44,45].