PART-B
CHAPTER 1

REVIEW OF LITERATURE
SECTION 1.1

Drug-Drug Interactions
1.1.1 Introduction

Recent advances in medicinal chemistry and pharmacology have resulted in the great plethora of new drugs being used in the therapy. These new chemical agents exhibit great potency and efficacy and their use as therapeutic agents has resulted in considerable progress in treating varied pathological conditions. However, these agents have also created alarming and growing clinical problems with drug-drug interaction (1). Serious drug-drug interactions have contributed to half of the recent market withdrawals of approved drugs and nonapprovals of a few new molecular entities in United States (2).

A drug-drug interaction may be defined as an unwanted change in the action of a drug due to previous or concomitant intake of another drug (3). The interactions can occur whenever a patient is administered two or more drugs simultaneously (4). In modern age therapy, a number of prevailing clinical situations require multiple drug therapy as a rule rather than the exception, thus involving concomitant administration of several drugs (5). A recent survey in United States indicated that usage of multiple drugs is very common. In a given week, when all medications including herbal supplements and over the counter drugs were considered, more than 81% of individuals would be taking at least one medication and more than 25% will be taking at least five medications. Furthermore, the highest prevalence of medications use was among women aged at least 65 years, of whom 12% took at least 10 medications and 23% took at least 5 prescription drugs (6). In such situations, chances of drug interactions become very high. These interactions, when occurring with widely available drug molecules are of growing interest and potentially growing worry for physicians as well as treated patients.

In recent years, interactions involving particularly substrates of the cytochrome P450 3A4 (CYP 450 3A4) isoforms have resulted in the withdrawal of drugs or restrictions in their use (7). Therefore, potential drug interactions should be searched with particular effort in cases where drug combinations are conveniently prescribed. **Table 1.1** describes drugs withdrawn from the United States market or not approved between 1998 and 2003 (2).
Table 1.1 Drugs withdrawn/not approved in United States market between 1998 and 2003

<table>
<thead>
<tr>
<th>Year</th>
<th>Withdrawn</th>
<th>Approval</th>
<th>Drug</th>
<th>Use</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>1998</td>
<td>1997</td>
<td>Bromfenac</td>
<td>NSAID</td>
<td>Acute liver failure</td>
</tr>
<tr>
<td>1999</td>
<td>1999</td>
<td>1988</td>
<td>Astemizole</td>
<td>Antihistamine</td>
<td>Drug-drug interaction, torsades de points</td>
</tr>
<tr>
<td>1999</td>
<td>1999</td>
<td>1997</td>
<td>Grepafloxacin</td>
<td>Antibiotic</td>
<td>torsades de points</td>
</tr>
<tr>
<td>1999(NA)</td>
<td>1999(NA)</td>
<td>Drug A</td>
<td>Drug A</td>
<td>Drug-drug interaction, torsades de points</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>2000</td>
<td>2000</td>
<td>Alosetron</td>
<td>Irritable bowel syndrome in women</td>
<td>Ischemic colitis, complications of constipation</td>
</tr>
<tr>
<td>2000</td>
<td>2000</td>
<td>1993</td>
<td>Cisapride</td>
<td>Heartburn</td>
<td>Drug-drug interaction, torsades de points</td>
</tr>
<tr>
<td>2000</td>
<td>2000</td>
<td>1997</td>
<td>Troglitazone</td>
<td>Diabetes</td>
<td>Acute liver failure</td>
</tr>
<tr>
<td>2001</td>
<td>2001</td>
<td>1997</td>
<td>Cerivastatin</td>
<td>Cholesterol lowering</td>
<td>Drug-drug interaction</td>
</tr>
<tr>
<td>2001</td>
<td>2001</td>
<td>1999</td>
<td>Rapacuronium bromide</td>
<td>Anesthesia</td>
<td>Bronchospasm</td>
</tr>
</tbody>
</table>

NA-not approved, b-reintroduced in market in 2002 with use restricted to patients severely affected with irritable bowel syndrome.

A drug interaction occurs when one drug modifies the activity of another, either enhancing or reducing its pharmacological effect. The outcome of such interaction may be beneficial if the therapeutic potency of the drug is enhanced, or harmful if the interaction causes an increase in adverse effects or reduction in efficacy.
of the drug. Beneficial effects resulting from the co-administration of drugs that are synergistic at their site of action can also result in an adverse drug reaction if the doses of drugs are not reduced to limit the risk of concurrent side effects. Conversely, if the drug interaction results in reduction in the activity of one or more of the co-administered drugs, then it may be necessary to increase the dose of the affected drug(s). If patients are taking multiple medications, there is always the option to discontinue one or more of the interacting medications. When an interacting drug is discontinued from a polytherapy regimen, the interaction goes into reverse and, depending on the underlying mechanism of drug interaction, may result in either reduced drug efficacy or enhanced toxicity (4).

In some circumstances, drug interactions are complicated and problematic. For example, interactions involving active metabolites of the co-administered drugs may not always be obvious if concurrent plasma concentration changes in the parent drug do not occur. It is not common practice to monitor plasma metabolite concentrations therefore, if one is unaware of the interaction; blood concentration monitoring of the parent drug could be misleading. However, awareness of the mechanism of drug interaction can be used to clinical advantage for example, when one drug reduces the rate of elimination of another and increases half-life (t1/2) of the affected drug, this can have impact on frequency of dosing, which in turn may improve compliance, or it may mean that a reduction of dose of the affected drug is necessary. In some circumstances, drug interaction may be considerable if it leads to economic benefits wherein, the cost of combined treatment is cheaper than that when treating with either drug alone. One example is the co-medication of valproate with lamotrigine, allowing dose of the latter to be reduced when these two anti epileptic drugs are used together (7).

In the past, relevant drug interactions were identified by empirical observation and physicians were often unaware of the potential for drug interaction or their importance. However, a more rational approach has been adopted in recent years with discovery and characterization of the enzyme systems responsible for drug metabolism, which are major targets for interference by concomitantly administered drugs. The development of in vitro screening assays for studying the interactions with CYP 450 enzyme system has allowed potential drug interactions to be anticipated before the drug reaches clinical stage of development. Consequently, in vitro screening for drug interactions is undertaken early which means that specific drug
interactions can be anticipated and looked for in early stages of drug development. More observations are made subsequently (4). The pharmaceutical companies have also started assessing clearance pathways and enzyme transporter modulating effects in early drug development stages (8-11).

1.1.2 Mechanisms of Drug Interaction

There are two types of drug interactions, pharmacokinetic and pharmacodynamic (4, 12). Pharmacokinetic interactions are those in which one drug interferes with the disposition of another and thereby alter the concentration of drug at the site of action (4). These interactions account for most of the interactions reported to date because they are easily identifiable by a change in drug concentration in the plasma.

Pharmacodynamic interactions are less well recognized and are commonly inferred to explain apparently drug-induced changes in clinical status that can not be attributed to a pharmacokinetic mechanism (13). These interactions take place at the cellular level where the drug acts and are usually not associated with changes in the plasma concentration of either drug (4).

1.1.3 Pharmacokinetic Interactions

Pharmacokinetic interactions can occur during absorption, distribution, metabolism, or elimination and are associated with drug-concentration changes in the plasma (4). These interactions can be further subdivided into those involving drug absorption, distribution and elimination (excretion or biotransformation) (3).

1.1.3.1 Interactions Affecting Absorption

Absorption is the entry of drug molecules into systemic circulation via mucous membranes of the gut or lungs, via the skin, or from the site of injection (4). It is the first point of interaction when two or more drugs are co-administered orally. The magnitude of response of drug is usually related to the circulating levels of active compounds, and these are controlled by the ease with which the drug passes from site of absorption into systemic circulation. Thus, interactions between drugs that influence absorption from the gastrointestinal tract (GIT) into the systemic circulation represent a major problem in drug therapy. Absorption of a drug from GIT depends on rate of dissolution, rate of gastric emptying, residence time of the drug in stomach
and transit time through the GIT. Transporters, particularly P-glycoprotein, may play an important part in the gastrointestinal absorption of many drugs including digoxin, cyclosporin, paclitaxel and docetaxel (14-19).

Absorption of drugs which are organic acids or bases is influenced by the gastrointestinal pH, depending upon where they exist as neutral molecules. Moreover, the pH of gastric fluids also influences gastric motility. High acidity inhibits emptying, while slight alkalinity enhances it (20). Antacids can interact with other drugs through their pronounced effect on gastric motility, gastric and urinary pH, and also by direct adsorption of drugs in solution (21). They can decrease as well as increase the absorption of other drugs from gut (22). Drugs in solution may be adsorbed on to insoluble agents such as kaolin, charcoal or cholestyramine. The bioavailability of cephalexin, sulphamethoxazole and thyroxine was found to decrease with cholestyramine (23). Some drugs may form insoluble salts or chelate complexes with metal ions. The effect of adsorbents becomes more complex if the drug undergoes significant amount of enterohepatic recycling (EHC) (24).

Alterations in the extent of drug absorption (F) give rise to a change in area under the curve (AUC), which precisely parallels the effects of altered dosage. Consequently, after a single dose, drug effects are increased or decreased depending upon the direction of change. During multiple dose therapy, changes in AUC described after single dose administration are reflected by AUC during a dosage interval. Inevitably, there is an appropriate change in concentration at steady state (C_{SS}), which is directly proportional to the change in the extent of absorption.

On the other hand, changes in the rate of absorption (k_a) do not lead to alterations in AUC either after a single dose or during multiple dosing. However, a slower rate of absorption delays the onset and reduces the intensity of a drug’s effect. These types of alterations are of special importance for those drugs where a rapid onset of action is desired for example, analgesics. Changes in absorption rate during multiple dose therapy do not influence mean C_{SS}, since the total AUC is unaltered, but do produce smaller fluctuations in plasma levels during a dosing interval. This is important for drugs like antibiotics and cytotoxic agents where transient high concentrations are required.
1.1.3.2 Interactions Affecting Distribution

Distribution is the movement of drug molecules between various water, lipid, and protein compartments in the body, including movement of drugs to their site of action, metabolism, and elimination. Interactions involving distribution of drugs are difficult to ascertain (4). These interactions may involve competition between two drugs for binding sites on plasma proteins. Several important pharmacokinetic properties such as hepatic metabolism rate, renal excretion rate, biomembrane permeation rate, tissue distribution and steady state distribution volume are functions of unbound drug concentration. The implications of plasma-protein displacement interactions are frequently misunderstood. The unbound (free) fraction of drug is in equilibrium with the receptor sites, and only this fraction has pharmacological effects. The amount of drug that is displaced from plasma proteins is generally a tiny fraction of the total amount of drug present in the body and is therefore insufficient to produce a change in the clinical response (25, 26). As a rule, displacement from plasma proteins results in a fall in total drug concentration as the displaced drug redistributes rapidly into the tissues and undergoes compensatory elimination, but the concentration of free drug and magnitude of the pharmacological effect are practically unchanged (27).

In quantitative terms, such interactions are more likely to be clinically significant when drugs are highly bound to the plasma proteins (>90%) and have small distribution volumes and narrow therapeutic window, because a small decrease in bound drug fraction will result in large increase in the fraction of unbound drug (28, 29). Therefore, awareness of these interactions is important for interpretation of plasma drug concentration measurements in clinical practice. In fact, in the presence of a plasma-protein binding interaction, therapeutic and toxic effects will occur at low total drug concentrations; patient management may benefit from the monitoring of free (unbound) drug concentrations (30, 31).

1.1.3.3 Interactions Affecting Elimination

Elimination is the removal of drug molecules from the body by excretion, usually by kidneys, or by metabolism (CYP 450 system), mainly in liver (4). The elimination rate of drugs is best described by Cl, which is analogous to renal clearance and describes the volume completely cleared of drug per unit of time. Most drugs are eliminated by renal excretion, metabolism, or combination of both and the
total clearance is equal to the sum of individual clearances for renal excretion and metabolism.

1.1.3.3.1 Interactions Affecting Excretion

Excretion is the process whereby drugs and/or their metabolites are irreversibly transferred from internal to external environment (32). Drugs that undergo extensive renal elimination in unchanged form may be susceptible to interactions affecting the excretion process, particularly when it involves active transport mechanisms or when the ionized state of drug is highly sensitive to changes in urine pH (33). The mechanisms by which two drugs can interact at renal excretion level include:

1. Competition for tubular secretion
2. Tubular reabsorption
3. Change in urinary pH due to any of the co-administered drug

An example of competition for the same active transport system in kidneys is increase in plasma concentration of penicillin by probenecid, which consequently reduces the renal excretion of penicillin (34). Urinary pH influences the ionization of weak acids and bases, thus affecting their reabsorption and excretion. An un-ionized drug more readily diffuses from glomerular filtrate into blood (24). An acidic drug is un-ionized more in acidic urine than in alkaline urine. Thus, it diffuses back into blood from acidic urine, resulting in prolonged activity. The examples include patients taking large doses of salicylates. The effects are opposite for a basic drug (e.g., dextroamphetamine). In one study, 54.5% of dose of dextroamphetamine was excreted within 16 h when the urinary pH was maintained at about 5, compared with 2.9% when the pH was maintained at about 8 (35). The other examples include increased clearance of chlorpropamide and decreased clearance of diethylcarbamazine by co-administration of sodium bicarbonate, which raises the urinary pH (24). Similarly, agents causing alkalization of urine increase the elimination of phenobarbital by reducing its reabsorption from renal tubuli, an effect that can be exploited therapeutically in severe cases of barbiturate intoxication (36).

1.1.3.3.2 Interactions Affecting Biotransformation

Biotransformation or metabolism of drugs is defined as the conversion from one chemical form to another (32). It is the most important mechanism of elimination
and drug interactions at metabolism levels are probably the most thoroughly investigated area. The biotransformation kinetics of one drug can be modified directly or indirectly by another drug in a number of ways, including induction and inhibition of drug metabolizing enzyme systems. These interactions might lead to loss of therapeutic activity or undue toxic reaction (37). A quantitative pharmacokinetic analysis of such interactions therefore requires that the time course of concentrations of both the drugs be monitored (38). The CYP 450 enzyme system are a major component of the mixed function oxidase system which are responsible for the metabolism of not only exogenous chemicals (xenobiotics), but also endogenous substances (e.g., corticosteroids) (4). The highest concentrations of these enzymes are found in liver and they catalyze majority of oxidative biotransformation (38). Characterization of isoenzymes involved in the metabolism of individual drugs and prediction of metabolic interactions has increased greatly in recent years (39-41). At the level of CYP, four isoenzymes (CYP3A4, CYP2D6, CYP2C9, and CYP1A2) are known to have role in the metabolism of 95% of all drugs, and 50-70% of all drugs might be substrate of CYP3A4 (42). The substrates, inducers and inhibitors of important CYP isoenzymes are described in Table 1.2 (27).

### Table 1.2 Substrates, inhibitors, and inducers of the major (CYP) isoenzymes involved in drug metabolism

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Substrates</th>
<th>Inhibitors</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Amitriptyline, clozapine, clomipramine, fluvoxamine, haloperidol, imipramine, mirtazapine, olanzapine, caffeine, theophylline, paracetamol, tacrine, tamoxifen, R-warfarin.</td>
<td>Ciprofloxacin, Clarithromycin, Fluvoxamine, Furafylline</td>
<td>Carbamazepine, Phenobarbital, Phenytoin, Primidone, Cigarette smoke, Charcoal-grilled meat, Rifampicin</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Phenobarbital, phenytoin, valproic acid, celecoxib, diclofenac, ibuprofen, naproxen, piroxicam, fluvastatin, losartan, tolfutamide, torasemide, S-warfarin, zidovudine</td>
<td>Valproic acid, Amiodarone, Chloramphenicol, Fluconazole, Fluoxetine, Fluvoxamine, Miconazole, Sulfaphenazole</td>
<td>Carbamazepine, Phenobarbital, Phenytoin, Primidone, Rifampicin</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Diazepam, S-mephenytoin, methylphenobarbital, phenytoin, amitriptyline,</td>
<td>Felbamate, Oxacarbazepine, Topiramate</td>
<td>Carbamazepine, Phenobarbital, Phenytoin</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>clomipramine, imipramine, citalopram, moclobemide, omeprazole, propranolol, proguanil, R-warfarin</td>
<td>Cimetidine, Fluoxetine, Haloperidol, Paroxetine, Perphenazine, Propafenone, Quinidine, Thioridazine</td>
<td>No inducer known</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td>Amitriptyline, citalopram, chlorpromazine, clomipramine, clozapine, imipramine, desipramine, fluoxetine, fluphenazine, fluvoxamine, haloperidol, mianserine, mirtazapine, nortriptyline, olanzapine, paroxetine, perphenazine, risperidone, thioridazine, venlafaxine, zuclopenthixol, alprenolol, bufuralol, encaidine, flecaidine, metoprolol, propafenone, propranolol, timolol, pindolol, codeine, debrisoquine, dextromethorphan, phenformin, tramadol</td>
<td>Cimetidine, Fluoxetine, Haloperidol, Paroxetine, Perphenazine, Propafenone, Quinidine, Thioridazine</td>
<td>No inducer known</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Felbamate, phenobarbital, dapsone, ethanol, halothane, isoniazid, chlorzoxazone</td>
<td>Disulfiram</td>
<td>Alcohol, Isoniazid</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Carbamazepine, ethosuximide, tiagabine, zonisamide, alprazolam, midazolam, triazolam, amitriptyline, clomipramine, clozapine, haloperidol, imipramine, sertraline, nefazodone, mirtazapine, risperidone, ziprasidone, olanzapine, amiodarone, atorvastatin, diltiazem, felodipine, lovastatin, nimodipine, nifedipine, quinidine, simvastatin, verapamil, alfentanil, astemizole, cisapride, clarithromycin, cyclosporin A, cyclophosphamide, erythromycin, fentanyl, glucocorticoids, itraconazole, ketoconazole, indinavir, sildenafil, tacrolimus, tamoxifen, terfenadine, oral contraceptive steroids</td>
<td>Cimetidine, Cyclosporin A, Diltiazem, Erythromycin, Fluconazole, Fluvoxamine, Grapefruit juice, Indinavir, Itraconazole, Ketoconazole, Nefazodone, Dextropropoxyphene, Ritonavir, Troleandomycin, Verapamil</td>
<td>Carbamazepine, Phenobarbital, Phenytoin, Primidone, Oxcarbazepine, Topiramate, Felbamate, Glucocorticoids, St John’s wort, Rifabutin, Rifampicin</td>
</tr>
</tbody>
</table>
These inducers are weaker or may induce CYP3A4 isoenzymes only in certain tissues.

1.1.3.3.2.1 Interactions Due to Enzyme Induction

Enzyme induction is increased synthesis of drug metabolizing isoenzymes in the liver and other tissues (43, 44). The most common mechanism for CYP induction is the de novo synthesis of new enzyme molecules as a result of a transcriptional activation (45). The increase in enzyme activity results in an increase in the rate of metabolism of drugs that are substrates of these enzymes and thus, the plasma concentration of the drugs is decreased. If the affected drug has an active metabolite, induction can result in increased metabolite concentration and possibly an increase in drug toxicity. As enzyme induction requires synthesis of new enzymes, the time course of induction and its reversal upon removal of the inducer is dependent on the rate of enzyme synthesis and degradation and the time to reach C_{SS} of the inducing drug. Thus, the time course of induction is generally gradual and dose dependent (43, 46, 47). One potent enzyme inducing agents known is the antitubercular agent rifampicin, which is used in treatment of tuberculosis, leprosy, and pleurisy. Rifampicin induces CYP3A4 and other CYP 450 enzymes in gut wall and liver in a dose and time dependent manner leading to numerous drug interactions with midazolam, triazolam, diazepam, oral anticoagulants, glucocorticoids, digitoxin, barbiturates and oral contraceptives (OCs) (48-55). The pharmacokinetic drug interactions of clinical significance involving antiepileptics and CYP-450 have been continuously reviewed (56-58). Drug interactions with tobacco smoking and alcohol may be clinically significant in case of theophylline, caffeine, tacrine, imipramine, haloperidol, pentazocine, propranolol, flecainide and estradiol (59, 60).

Presently, herb-drug interactions have gained considerable attention. St. John's wort (Hypericum perforatum) is a dietary supplement often used for depression (61). Recent in vitro studies indicate that hyperforin; one of the main components is a potent inducer of CYP3A4 enzyme (62). The extracts increase metabolism of various drugs, including combined OCs, cyclosporin, and indinavir, via induction of pregnane-X receptor, a nuclear receptor that regulates expression of the CYP3A4 monooxygenase, in human hepatocytes (62).
1.1.3.3.2.2 Interactions Due to Enzyme Inhibition

Enzyme inhibition is the phenomenon, by which a drug or its metabolite block the activity of one or more drug metabolizing enzymes, which results in a decrease in the rate of metabolism of the affected drug. This in turn will lead to high plasma concentrations of the drug and possibly clinical toxicity. Enzyme inhibition is normally competitive and dose dependent, and begins as soon as sufficient concentrations of the inhibitors are achieved. Substantial inhibition is seen within 24 h of the inhibitor being given in many cases (40, 41).

Significant metabolic interactions involving enzyme inhibition include drug interactions with grapefruit juice. Grapefruit juice inhibits the intestinal CYP3A4 system, and increases the AUC by 1250 to 1400% from the baseline values for lovastatin and simvastatin respectively from a sample of studies (63, 64-74). Through the inhibition of this enzyme system, grapefruit juice interacts with a variety of drugs, leading to elevation in serum concentration of these drugs (75). Clinically relevant interactions are more likely with dihydropyridines, terfenadine, saquinavir, cyclosporin, midazolam, triazolam and verapamil and may also occur with lovastatin, cisapride, astemizole, artemether, carbamazepine, amidorone, cisapride, felodipine and buspirone (73, 76-82).

Inhibitors of hepatic microsomal oxidation like phenylbutazone, disulfiram, chloramphenicol, phenothiazines, etc. might precipitate the toxicity of many drugs with a narrow therapeutic window like warfarin and phenytoin. The drug interaction potential of well-known inhibitors like cimetidine and ranitidine have been reviewed earlier (59, 83). Among the antimicrobial agents, enzyme inhibition is well known with macrolide antibiotics like erythromycin and triacytyleoleandomycin, latter being the more potent one (84, 85). Antifungal agents of azole group e.g., ketoconazole and fluconazole are broad-spectrum inhibitors of CYP 450 and interact with a number of drugs (85). The fatal interaction between ketoconazole and terfenadine was due to increased terfenadine levels by virtue of enzyme inhibition property of ketoconazole (86).

1.1.4 Pharmacodynamic Interactions

Pharmacodynamic interactions take place directly at the site of action and result in a modification of pharmacological effect. The effects of interacting drugs can be additive, synergistic, or antagonistic (87). Pharmacodynamic interactions can
be adverse when the increase in toxicity is greater than any gain in drug activity or beneficial when therapeutic effects are additive or synergistic and toxic effects are less than additive. To some extent, these interactions can be explained through knowledge of the mechanisms of action of individual drugs. For example, the similarity in mechanisms of action between carbamazepine and oxcarbazepine may explain why neurotoxic effects are more common when oxcarbazepine is used with carbamazepine than when used with any other antiepileptic drug (27).

1.1.5 Steroidal Contraceptives and Drug Interactions

Since their introduction in 1960s, steroidal OCs have become among the most widely used pharmaceuticals. Approximately 60 to 100 million women worldwide take the OC pill (88). OCs represents a unique group of drugs in therapeutics as they are used by a predominantly healthy population (89). OCs remains one of the most effective reversible methods of birth control available today, providing almost 100% effectiveness with an impressively high margin of safety and other important health benefits (90). However, in cases where a pregnancy occurs during OC use, the blame is usually laid against the user for having forgotten the pill or against the pill itself. Evidences suggest that the efficacy failure may be because the user is taking other medicines, which may be interacting with the OCs (91). There have been reports on drug interactions with OCs in which few are of definite therapeutic relevance, whereas others can be discounted as being of no clinical significance (92).

Pharmacokinetic data of ethinylestradiol (EE), the major estrogenic component of combined OCs indicates that it is well absorbed after oral administration but is subjected to first pass metabolism. Oral bioavailability averages 38-48%, with greater inter-individual variability (20-65%) (93). EE is 97% bound to serum albumin and undergoes aromatic 2-hydroxylation during metabolism accomplished largely by CYP3A4. This metabolite is further methylated and conjugated with glucuronic acid before urinary and fecal excretion. The conjugates of EE secreted in the bile may get hydrolyzed by gut bacteria to liberate the parent compound, which can undergo enterohepatic circulation (EHC) (94). The elimination t1/2 of EE varies between 13 and 27 h at steady state (95).

Levonorgestrel is rapidly and almost completely absorbed after oral administration. Levonorgestrel undergoes reduction and hydroxylation in liver,
followed by conjugation with sulfate and glucuronic acid. The elimination \( t_{1/2} \) of levonorgestrel is variable, averaging \( 36 \pm 13 \) h at steady state (96-98).

Norgestimate is well absorbed after oral administration and is partly converted to an active, deacetylated derivative with \( t_{1/2} \) of 12-30 h. Other hydroxy metabolites and conjugates are also formed (99). Norethindrone (acetate) is well absorbed, with a mean bioavailability of 65% due to presystemic clearance. It chiefly undergoes reduction followed by conjugation with glucuronide or sulfate. Elimination \( t_{1/2} \) of norethindrone exhibit considerable variability (5-14 h). Norethindrone acetate is completely and rapidly deacetylated to norethindrone after oral administration (96). Ethynodiol acetate is also quantitatively metabolized to norethindrone (96).

Desogestrel is rapidly absorbed, with an oral bioavailability of 84%. The parent compound is converted to an active metabolite, 3-keto-desigesterl, which is 99% bound to sex hormone-binding globulin (SHBG) and has a elimination \( t_{1/2} \) of 28-31 h. With exception of levonorgestrel, other progestins are not subject to direct conjugation. They are extensively reduced to inactive compounds before conjugation. Ethinyl estradiol and all progestins used in OCs are subject to large inter-individual variations in disposition (99).

An overview of the drug interactions reported with OCs reveals that the mechanism by which these interactions occur predominantly involve either enzyme induction/inhibition or the possible disruption of EHC of estradiol component (92). Two general classes of drug interactions involving OCs have been reported: (i) those that may impair the efficacy of OC steroids, leading to breakthrough bleeding and pregnancy or the enhancement of activity of the contraceptive; (ii) those where OCs may interfere with the metabolism or activity of other therapeutic agents (89). The latter part is not discussed, as it is not relevant here.

1.1.5.1 Interactions with Antibiotics

The first report of interactions between OCs and antibiotics appeared in 1970s when rifampicin was implicated in unwanted pregnancies and frequent bleeding in OC users (100). Since then, reports of pregnancies have been observed in OC users receiving ampicillin, tetracycline, chloramphenicol, griseofulvin, sulphonamides and others (101). The postulated interaction potential of broad-spectrum antibiotics was reviewed by Weaver and Glasier (102). Based on literature, they concluded that
rifampicin and griseofulvin have genuine interaction with OCs leading to reduced efficacy.

Antitubercular drug rifampicin is well known for its CYP 450 enzyme inducing property. The induction of enzymes involved in 2-hydroxylation of ethinylestradiol, especially CYP3A4 is one of the major targets (103). With the emergence of multiple drug-resistant tuberculosis and tuberculosis in HIV/AIDS patients, the drug regimens have become more complex thereby augmenting the potential of drug interactions (104). There have been numerous reports on pharmacokinetic interactions of rifampicin with OCs revealing a significant reduction in bioavailability of estradiol component (52-54, 105). The interaction potential of rifamycin, rifabutin vis-à-vis that of rifampicin was recently evaluated by Barditch-Crovo et al., where rifampicin was found to be more significant inducer of ethinylestradiol and norethisterone clearance than rifabutin (106).

In the area of broad-spectrum antibiotics, situation appears to be less clear. Although interactions have been continuously published by medical journals, small number and retrospective nature of studies make it hard to draw a concrete conclusion. Antibiotics like ampicillin, sulphonamides and tetracycline may interact with OCs via destruction of normal gastric flora responsible for breakdown of estradiol conjugates in intestine disrupting their EHC (107,108). The chances of interaction with low-dose long-term administration of antibiotics are less likely especially if resistant gut flora rapidly emerge and re-establish the EHC of steroid hormones (109). Moreover, it has been postulated that high risk of interaction is associated with a subset of women who have lower bioavailability of ethinylestradiol, a significant EHC and gut flora susceptible to the antibiotic being used (110). Therefore, cautious approach of using additional means of contraception during short courses and initial weeks of long-term courses of broad-spectrum antibiotics is justified because of serious consequences of unwanted pregnancy (102). Some of the macrolide antibiotics can also interact with OCs, usually by altering metabolism via inhibition of CYP-450 in liver, by disturbing gastric flora or through enhanced gastric emptying due to motilin like effect (111).

1.1.5.2 Interactions with Anticonvulsants

Over 10% of women of reproductive age have experienced a serious chronic disorder of which, epilepsy is among the most common neurological problem (112).
The anticonvulsants have long been implicated to interact with OCs. This is due to the prevalence of epilepsy in women of reproductive age; long-term therapeutic requirement and induction effect of most anticonvulsants on hepatic microsomal enzymes, particularly CYP3A4 isoenzyme. This results in acceleration in the hydroxylation of estrogen to inactive metabolites and decreasing its serum concentration thereby causing efficacy failure. Phenytoin, phenobarbital, carbamazepine and primidone have been reported to cause contraceptive failure, whereas valproic acid does not diminish the activity of OCs (113-118). Among the second-generation anticonvulsant drugs, febamate, oxcarbazepine, and topiramate may reduce efficacy of OCs (119-121). Although reduced efficacy of OCs is generally attributed to an effect on estrogen component, reduction in progestogen due to anticonvulsant therapy have also been associated with contraceptive failure (122, 123). The teratogenicity of anticonvulsants further makes the failure of contraception a serious issue. The use of anticonvulsants has extended to affective disorders, migraine and pain, thus increasing the risk of drug interactions. Anticonvulsants also stimulate production of SHBG thereby increasing the binding of contraceptive steroids and decreasing circulating levels of free steroid. Therefore, alternative contraceptive measures are recommended to avoid unwanted pregnancies.

1.1.5.3 Miscellaneous Interactions

Vitamin C and acetaminophen give rise to increased blood concentrations of ethinylestradiol, due to competition for sulphation. The interactions have some significance to women on OCs that regularly take high doses of either drug. Although adsorbents like magnesium trisilicate, aluminium hydroxide, activated charcoal and kaolin can interfere with OC efficacy, there is no firm evidence to support this. Smoking is also believed to alter the pharmacokinetics of OC steroids but lacks concrete evidence (63).

1.1.5.4 Clinical Significance

There is ample literature available on drug interactions with OCs, although their incidence is not known. The potential clinical significance of some interactions makes it important for all prescribing doctors to be aware of these interactions. It is now a well established fact that impairment of the OCs is most likely to be due to interference with the estradiol component as it undergoes sulphation in gut,
hydroxylation and glucuronidation in liver followed by enterohepatic recirculation. Therefore, OCs are affected by enzyme inducing agents viz. rifampicin, griseofulvin and anticonvulsants and the clinical significance of these interactions has been well-established (109).

On the other hand, there is considerable variation in opinion about the importance of drug interactions between OCs and other broad-spectrum antibiotics. There are relatively few prospective studies of the pharmacokinetic interaction of antibiotics with OCs and few demonstrate a convincing basis for any reduced contraceptive efficacy. There is evidence however, that variability in handling of OCs could render some women more susceptible to OC failure.

Due to lack of prospective, well designed pharmacokinetic or population based studies concerning the drugs in question, the perceptions regarding the drug interactions with OCs are largely based on anecdotal evidence. Among scientific groups, which systematically assess clinical evidence, anecdotal data are usually considered as non-evidence. Consequently, without quality evidence, there are varying and conflicting views as to how to manage the co-administration of drugs with OCs. However, considering the serious consequences of unwanted pregnancy, cautious approach of using additional contraceptive measure or non-hormonal contraceptives, short-term use of higher dosage of OCs or, for long-term application, continuous administration of monophasic micro-pill preparations is well justified during concomitant use of antibiotics or anticonvulsants (124).

The current situation of conflicting opinion and advice is potentially confusing to both doctors and patients. In such a situation the health professionals who prescribe OCs must continue to strive to educate women about the times when there is greatest danger of failure. The doctors and dentists who prescribe antibiotics to women, are required to enquire specifically about OC use, and if necessary give advice about possible interaction (104). Despite of varying and conflicting views associated with drug interactions with OCs, the potential threat posed by these drug interactions and subsequent outcome cannot be overlooked. A careful and detailed dissection of these interactions is, therefore, necessary with newer combinations of drugs.
1.1.6 Nonsteroidal Contraceptives and Drug Interactions

There is paucity of information on data regarding drug interactions of nonsteroidal and other post-coital contraceptives. There is one but conclusive report on inactivation of CYP 450 by mifepristone and its analogs (125). Among antiestrogens having non-contraceptive uses, the most thoroughly investigated drug is tamoxifen as far as drug interactions are concerned. Since the therapeutic use of tamoxifen is specifically concentrated on breast cancer and it forms a part of multiple drug therapy, the potential of drug interactions is substantially high. Eventually, there are number of reports, which evaluate interaction potential between tamoxifen and other anti-cancer drugs, viz. etoposide, doxorubicin and aromatase inhibitors (126-128). While there is no pharmacokinetic interaction with etoposide and doxorubicin, the aromatase inhibitors do interact with tamoxifen. In rats, the aromatase inactivator 4-hydroxy androstenedione, which is given concurrently with tamoxifen to counteract the potential tumor resistance to tamoxifen, inhibits hepatic CYP 450. Thus, it could potentiate efficacy of tamoxifen and diminish side effects due to formation of reactive intermediates during metabolism (129).

The interactions have also been reported between tamoxifen and phenytoin, possibly at metabolic site, while treatment of seizures related to brain tumors, with mitomycin C leading to hemolytic uremic syndrome, with allopurinol increasing its hepatotoxicity and with theophylline resulting in a significant decrease in $t_{1/2}$ of theophylline (130-133). Life threatening interactions of tamoxifen with warfarin have been reported with possibility of interaction via reduction in metabolism of warfarin due to tamoxifen rather than the protein binding displacement (134, 135). In another study, effect of rifampicin co-administration on pharmacokinetics of tamoxifen and toremifene was reported in which, rifampicin significantly reduced the AUC, concentration maxima ($C_{\text{max}}$) and elimination $t_{1/2}$ of both tamoxifen and toremifene by around 85%, 55% and 45%, respectively (136).
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SECTION 1.2

Research Envisaged
1.2.1 Introduction

DL-Centchroman (INN: Ormeloxifene) synthesized at the Central Drug Research Institute, Lucknow, is a non-steroidal once a week OC (1, 2). Centchroman (CC) was introduced in Delhi in July 1991 and included in the National Family Welfare Programme in 1995 (3-6). It is a unique need-oriented contraceptive being effective when taken immediately after coitus or routinely as a weekly pill (2, 5-17). Table 1.3 describes some important chemical parameters of CC (18, 19-22).

Table 1.3 Compound data sheet of CC

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Appearance</th>
<th>M. P.</th>
<th>Solubility</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-1-[2-{4-(7-methoxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran-4-yl)-phenoxy}-ethyl]-pyrrolidine hydrochloride</td>
<td>White crystalline solid</td>
<td>165-166°C</td>
<td>Soluble in chloroform, acetone, methanol and ethanol</td>
<td>Highly stable under normal storage conditions</td>
</tr>
<tr>
<td>CH₃O</td>
<td>M.P. 165-166°C</td>
<td>Soluble in chloroform, acetone, methanol and ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃O</td>
<td>Soluble in chloroform, acetone, methanol and ethanol</td>
<td>Highly stable under normal storage conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃O</td>
<td>Soluble in chloroform, acetone, methanol and ethanol</td>
<td>Highly stable under normal storage conditions</td>
<td></td>
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</tr>
</tbody>
</table>

1.2.2 Pre-clinical Pharmacokinetics

Distribution and excretion pattern of [¹⁴C]-CC after 1.25 mg/kg oral and intravenous administration in female rats and rhesus monkeys are reported (23, 24). After intravenous administration in rats, 15.6% of the administered dose was observed in blood in initial 2 min and then it gradually declined to 0.43% at 120 h. Following oral administration, CC concentration increased exponentially with time in most of the tissues with maximum radioactivity observed between 8 or 24 h. The excretion was mainly through feces accounting for 82% of the administered dose while 4% of radioactivity appeared as ¹⁴CO₂ and very little was observed in urine over the 21-day study period. Well-perfused organs (liver, heart, lung and intestine) retained more radiolabel compound than the less perfused organs (genital organs, thyroid, fat and...
adrenal gland) (23). In rhesus monkeys treated with $[^{14}\text{C}]-\text{CC}$ orally, total radioactivity in examined tissues increased exponentially with time and attained maxima at 4 h followed by exponential fall. The major route of excretion was through feces, which accounted for 70% of the administered radioactivity. Relatively low levels were detected in established estrogen target tissue, while high amounts of radioactivity were found in organs normally not considered as estrogen target tissues such as liver, lung, and pancreas (24).

Pharmacokinetics of unlabeled CC has been studied in female Sprague-Dawley rats at 12.5 mg/kg and 1.5 mg/kg single oral dose (25, 26). Following 12.5 mg/kg oral administration, $C_{\text{max}}$ for CC in plasma were observed at 4 and 12 h while its active metabolite, 7-desmethyl centchroman (7-DMC) exhibited $C_{\text{max}}$ at 8 and 24 h post dose. $C_{\text{max}}$ for CC occurred between 8-12 h in all tissues, while its active metabolite 7-DMC exhibited $C_{\text{max}}$ between 8 and 24 h post dose. Tissue to plasma ratio of CC was highest in lungs (>200), followed by spleen, liver, adipose tissue and uterus (>40) while for 7-DMC the ratio was highest in lung (>350) followed by liver, spleen, uterus (>28) and adipose tissue. Terminal $t_{1/2}$ of CC and 7-DMC in rat plasma was 24.1 and 36.6 h, respectively. Mean residence time (MRT) of CC and 7-DMC was highest in liver followed by uterus and it was lowest in plasma for both the drug and the metabolite (26).

Following 1.5 mg/kg oral administration in rats, two $C_{\text{max}}$ for CC and 7-DMC were observed at 1 and 18 h, respectively. Portal-venous, biliary and fecal excretion studies indicated discontinuous absorption along GIT as the probable reason for observed multiple peaks. However, double peak phenomenon exhibited by 7-DMC was attributed to EHC. Excretion of CC up to 120 h was found to be ~16% as parent and 10% as 7-DMC of the administered dose in feces (25).

1.2.3 Clinical Status

CC has been reported to provide good pregnancy protection in women in post-coital as well as weekly regimen (8). It was introduced as a contraceptive pill in 1991 and is commercially available as Saheli (M/s Hindustan Latex Ltd., India) and Centron (M/s Torrent Pharmaceuticals Ltd., India). The post marketing surveillance of 1994 reports a success rate of more than 99% in the users (10). CC and its tablet formulation are listed in Indian Pharmacopoeia with a categorical use of oral contraceptive (21).
A total of 1,957 parous women of reproductive age group have been covered for over 21,000 months of use with excellent pregnancy protection without any side effects. The recommended dosage of CC in humans as an OC is 30 mg twice a week for first 12 weeks followed by 30 mg weekly as long as contraception is desired. This regimen is reported to have the Pearl index of 1.83 and life-table analysis of 1.63±0.74 at 12 months. The use of CC is devoid of any side effects except prolongation of 8% of the menstrual cycles. The contraceptive effect is readily reversible and subsequent pregnancy is normal (7).

Pharmacokinetics of CC has been studied in normal healthy females after single oral dose of 30 and 60 mg, and also after multiple oral doses of 30 mg twice a week for 12 weeks (27-29). The single dose study revealed that pharmacokinetics of CC follows a two-compartmental model. It is slowly absorbed, widely distributed and slowly eliminated from body. The comparison of two studies showed that absorption and disposition of CC are reproducible, dose-independent and follow first order kinetics while C\text{max} and AUC\text{0-\infty}, were dose-dependent. The elimination t\text{1/2} in both studies was found to be around 7 days. Pharmacokinetics of CC in nursing women and its excretion into breast milk has been established in healthy female subjects (30-32). The study indicated that a maximum infant dose of 11% of the maternal dose per week and less than 2% of the maternal dose per day received would not infer any physiological consequences to breast-fed infants.

Apart from contraceptive use, CC is also effective against advanced breast cancer (33). CC has indicated prevention of bone loss due to osteoporosis in laboratory animals and postmenopausal women (34, 35). It could also be effective in the treatment of dermatitis, including eczematous dermatitis and psoriasis (36).

1.2.4 Problem and its Importance

Today, CC is used by women of reproductive age group as an OC and is likely to be used in future for a variety of other ailments, such as breast cancer and osteoporosis by women of all age groups. It will be used by healthy women as well as women suffering from acute and chronic ailments, irrespective of demography. Considering widespread and long-term use of CC in a number of clinical situations, the concurrent use of other drugs is imperative. Under these circumstances, the possibilities of drug interactions are no exception. This may, in turn affect the
efficacy of CC and therefore needs thorough evaluation in order to achieve the ultimate goal of "safe and rational use". In view of the interactions observed with the hormonal OCs, such studies become more important.

As emphasized earlier, interaction studies have become an integral aspect of drug development programme. Studies of all possible interactions are neither practically viable nor economic; therefore, careful selection of a limited number of drug combinations is essential. Based on these principles, the present study was planned with an aim to generate a "Pre-clinical drug-drug interaction database" for CC in animal (rat) model. The pharmacokinetic profile of CC was generated in rats which served as control for comparison. The drug interaction studies evaluated the impact of oral co-administration of selected drugs on pharmacokinetic profile of CC and subsequently pharmacological (anti-implantation) activity of CC together with the co-administered drug(s) was tested.

The co-administered drugs were selected on the basis of frequency of clinical use in target and likely population of CC users. On this basis, the drugs from different categories viz., antibiotics (ciprofloxacin, cefixime, amoxicillin and metronidazole), antihypertensives (amlodipine, atenolol), antiasthamatic (theophylline) and antidiabetics (metformin, pioglitazone and glibenclamide) were screened for their interaction potential with CC.
References


