COMPARISON OF THE EFFECTS OF CIMETIDINE AND TRAMADOL ON THE DISPOSITION OF PARACETAMOL IN HEALTHY HUMAN VOLUNTEERS

BY

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MSc/Pharm. Sci/23574/2000-01

AUGUST, 2006

DECLARATION

THIS IS TO CERTIFY THAT THE WORK REPORTED IN THIS THESIS WAS CARRIED OUT BY ME UNDER THE SUPERVISION OF DR. M. GARBA AND DR. (MRS) M.T. ODUNOLA IN THE DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY, FACULTY OF PHARMACEUTICAL SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA.

THE WORKS OF OTHER INVESTIGATORS WERE ACKNOWLEDGED AND REFERRED TO ACCORDINGLY. I DECLARE THAT NO PART OF THIS THESIS HAS BEEN SUBMITTED ELSE WHERE FOR THE PURPOSE OF A DEGREE.

GAMAWA ADAMU IBRAHIM

CERTIFICATION

This thesis entitled “COMPARISON OF THE EFFECTS OF CIMETIDINE AND TRAMADOL ON THE DISPOSITION OF PARACETAMOL IN HEALTHY VOLUNTEERS” BY GAMAWA ADAMU IBRAHIM meets the regulations governing the award of the degree of Master of Science (Pharmaceutical Chemistry) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

For courage and patience. For Shafiu, who advised me to be a pharmacist.

ACKNOWLEDGEMENT

Glory be to Allah (S.W. T) who made this work possible and may Allah pour salutation on our nosle prophet Muhammad (S.A. W) for bringing the guidance we all cherish as human beings. I am most grateful to my late parents who laid the foundation for me.

I am also grateful to my project supervisors Dr. Magaji Garba and Dr. (Mrs) Odunola Bakari Moji for their valuable contributions, encouragement and guidance.

My gratitude also goes to friends and relations who contributed immensely to this project with prayers and support, but are too numerous to mention here but I must make mention of Zara'u Bello who took care of the home front in my absence. I also have to mention Pharm Ahmed Gana of AKTH Kano without whose contribution this work would have remained a dream. I am also grateful to Abdullahi Abdulhamid my most devoted and selfless volunteer and to all the volunteers. The contributions and special interest shown by Alhaji Hamza Sukwa is well appreciate.

I am also grateful to all members of staff and Academic Staff of Pharmaceutical Chemistry especially Mal' Iliyasu and the faculty members at large.

Last but by no means the least my gratitude goes to His Excellency Amb. Uche Okeke, who provided me with the time and material needed for this study and to my immediate boss Dr. L. Idris who recommended me for such a privilege.

ABSTRACT

The influence of cimetidine (400mg) and Tramadol (100mg) on the disposition of oral single dose (1g) Paracetamol was studied and compared in 8 healthy human volunteers under two protocols; concomitant and delayed paracetamol administration.

Subjects received one treatment at each of five sessions 2wk apart. Ingestion of Paracetamol (1g) alone was used as control and compared with paracetamol concentration on concomitant (with tramadol (100mg) and then cimetidine (1g)) and delayed administration (1h 45mins after tramadol and 1h after cimetidine). Saliva paracetamol concentrations were determined with UV-Spectrophotometer. Pharmacokinetic parameters (kab, t1/2ab, kel, t1/2el and lag time), were determined by plotting paracetamol concentrations against time on a logarithmic scale while other parameters were calculated.

For the influence of cimetidine and tramadol on paracetamol when either of the drugs was administered concomitantly with the paracetamol there were no significant changes ($P>0.05$) when compared to the control.

On delayed administration of paracetamol 1h 45mins after tramadol however, Tmax and t1/2ab were significantly ($P<0.05$) increased by 20.21% and 19.47% respectively while kab was significantly ($P<0.05$) reduced by 25.88%. The other parameters were not significantly affected ($P>0.05$).

Delayed administration of paracetamol 1hour after cimetidine on the other hand affected all the pharmacokinetic parameters significantly. The (Cmax) and kab were significantly reduced ($P<0.05$) by 31.38% and 50.24% respectively, while Tmax, $t^1/2ab$ and lag-time were significantly increased ($P<0.05$) by 45.65%, 46.65% and

36.41% respectively. The $t^1/2el$ and AUCo-∞ were also significantly increased ($P<0.50$) by 59.31% and 29.45% respectively. Clearance (cl) and kel on the other hand were significantly reduced ($P<0.050$) by 21.90% and 59.74% respectively. The volume of distribution (vd) was significantly increased by 47.30%.

These findings indicated that cimetidine and tramadol do not affect the pharmacokinetics of paracetamol when either drug was taken concomitantly with paracetamol. However, when paracetamol is administered 1h 45mins after tramadol (100mg), there was a significant delay in the absorption of paracetamol, but the elimination is not significantly affected. There is both impaired absorption and inhibited metabolism of paracetamol when the administration was delayed 1 hour after cimetidine.

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CHAPTER ONE

INTRODUCTION

1.0 DRUG INTERACTIONS

Klaus and Jouni, (2001) stated that when two or more drugs are given together, the response may be greater or smaller than the sum of the effects of the drugs given separately. They further said that one drug may potentiate or antagonise the effects of the other and in some cases there may also be qualitative difference in their response. Doctors have always practiced poly pharmacy and a sound combination of drugs help to increase the efficacy and safety of drug treatment.

The true prevalence of undesirable drug interactions is substantial but largely unknown. It has been estimated that the number of death attributed to adverse drug reactions may be as high as 200,000 deaths per year in the United States (Chyka, 2000). A pharmacoepidemiological study conducted by Kennedy et al, (2000) demonstrated that half the population of 1225 adult general surgical patients were taking medicines that were not related to surgery. On average these patients received nine different drugs which may interact. The Boston collaborative drug surveillance programme reported a study of 9,900 patients with 83,200 drug exposures and found 3,600 adverse drug reactions, 234 (6.5%) of which were attributable to drug interactions. In a study where the medical charts of 1,800 surgical patients were reviewed researchers found at least one potential drug interaction in 17% of patient (Durrence et al, 1985).

Many adverse drug interactions are the result of concomitant therapy with potent drugs. Patients treated with phenothiazines, corticosteroids, antineoplastics and many

other drugs must frequently be subjected to certain adverse effects in order to obtain therapeutic benefit.

A possible correlation has been noted between the significant increases in adverse effects and the use of multiple drug therapy (Evaluation of drug interaction, 1997). The incidence of drug reaction increased with the number of drugs prescribed simultaneously, and drug interaction make a small but significant contribution to the overall morbidity and mortality due to drugs.

Many of the drug interaction reported in the literature are anecdotal and have not been confirmed, nor does there exist any well pharmacological basis for believing they could occur (Griffin and D'Arsy, 1979). Nevertheless, individual variability is such that factors like pharmacokinetic differences and effects of disease states may have contributed to a unique reaction. Environmental factors such as smoking and atmospheric pollution or even the hardness of the water supply have also been reported to influence drug metabolism and may also be involved in contributing to a drug interaction. Other causes are dietary factors and particularly herbal remedies which there is increase in their usage by the population due to the mistaken belief that they are free of adverse effect, when in fact their usage in surrounded by ignorance of their pharmacology and toxicology (Dukes, 1973). Outside the hospital less information is available on the multiple usage of drugs. Patients frequently use over the counter drugs (OTC), which they prescribed for themselves along with the doctor's prescription. These drugs frequently interact thereby complicating drug treatment.

In the light of these informations it seems reasonable to speculate that a very large number of patients may be at risk of having potentially harmful drug interaction and that an important problem in modern therapeutics might exist (Graham, 1977)

1.1 MECHANISMS OF DRUG INTERACTIONS

Drugs may interact in a number of ways such as pharmaceutical, pharmacodynamic or pharmacokinetic basis. A number of drugs may also interact at several different sites. Such cases represents drug – drug interactions. Other forms of interactions also do occur. Therefore, the concept of drug interactions also include the modification of drug effects by food or dietary items (drug – food interactions). There may also be drug – laboratory test interaction, where a drug causes alteration of diagnostic laboratory test.

Drug – disease interaction represents a situation where some drugs are contra-indicated in certain disease states.

1.1.1 Pharmaceutical drug interactions

Pharmaceutical interactions normally occur before the drug in given to the patient. They may be caused by several different mechanisms. When injecting for instance thiopentone with vecuronium through the same giving set, a precipitate will form instantaneously. Numerous incompatibilities have been demonstrated, and drugs should never be mixed in this fashion unless the absence of reaction has been clearly established (Trisel et al, 1994).

Pharmaceutical interactions can also be described as physicochemical incompatibilities, which are unintentional interactions that occur in vitro between drug and other component of medicinal products during their preparation, storage or

administration. Drug-drug, drug-excipient, excipient-excipient, drug-packaging, and excipient-packaging are all interactions that may cause adverse effects on bioavailability, efficacy or toxicity.

Another important area of drug interaction of clinical important occurs when drugs are added to intravenous infusion. Over the last ten years the practice of administering drug by continuous intravenous infusion has become more common, particularly in surgical unit. The problem is mainly produced by incompatibility between the drug added and component of the infusion fluid (Kramer, Inglott and Cluxton, 1974).

1.1.2 Pharmacokinetic drug interactions.

Pharmacokinetic drug interactions can be divided into interactions that occur during absorption, distribution or elimination. In some cases, drugs may interact simultaneously during several different phases during passage of the drug through the body.

Absorption interaction

Drugs can influence the absorption of other drugs at least by changing the gastrointestinal pH and motility, by intraluminal binding or the chelation of drug, by changing the regional blood flow, by inhibition or stimulation of first pass metabolism, or through toxic effects on the gastrointestinal canal. The subcutaneous and intramuscular absorption of drugs can be delayed or decreased after the administration of drugs affecting regional blood flow (vasoactive agents). However, these mechanisms have no major importance in pharmacokinetics. (Klaus and Jouni, 2001).

Table 1.1.2.1 possible mechanisms of drug absorption interactions

- pH effect on dissolution and ionization
- Changes in gastric emptying and GI motility
- Formation of complexes, ion pairs and chelates
- Interference with active transport
- Disruption of liquid micelles
- Changes in portal blood flow
- Toxic effect on G.I mucosa
- Change in volume, composition and viscosity of secretion
- Effects on mucosal and bacterial drug metabolism
- Change in membrane permeability

Because the rate of absorption of orally administered drugs is directly proportional to the rate at which drugs pass from the stomach to the intestine, some drugs or agents by increasing gastrointestinal motility, may increase the rate at which another drug passes through the gastrointestinal tract which could lead to an increase in absorption. Conversely, muscarinic receptor blockers e.g. atropine will reduce gastrointestinal motility, decreasing dissolution of drugs in the GIT and consequently absorption. This may lead to increase in plasma levels of drugs due to prolong contact with the absorptive surfaces (Nimmo et al 1973) show that propantheline and metochlopramide delay and accelerate gastric emptying respectively.

Table 1.1.2.2 Drugs that might influence gastric emptying.

- Atropine and anticholinergics
- Antihistamines
- Tricyclic antidepressants

- Phenothiazines
- Sympathomimetics
- Antiparkinson drugs
- Narcotic analgesics
- Nitrites
- Iproniazid
- Chloroquine
- Metoclopramide
- Caffeine
- Prostaglandins
- Antacids
- Antihypertensives

Drugs are probably absorbed more rapidly from the upper small intestine than from stomach because of the much greater surface area of the intestine (Levine, 1970). The rate of gastric emptying may therefore limit the rate of drug absorption and is very important in the context of drug interactions since it can be influenced by many drugs (Morselli et al, 1974). Because of this, any factor influencing gastric emptying and therefore drug movement into the small intestine will alter the rate and possibly the extent of absorption.

Changes in GIT pH also affect the absorption of most drugs. Since many drugs occur as acids or bases or contain more than one ionisable group it is expected that PH of the stomach could influence the extent of their absorption. Drugs cross membranes more readily in their un-ionised form due to higher lipophilicity of this species. According to the pH – partition theory, weak organic acids are largely absorbed from the stomach where as weak bases are absorbed best from the more alkaline contents of

the upper small intestine (Brodie, 1964). The absorption of weak acids is reduced if they are given with alkali since fewer drugs would be present in the un-ionised lipid soluble diffusible state (Binns et al, 1971).

However, an opposite effect may be observed. Aspirin, for example is absorbed more rapidly from buffered alkaline solution than from unbuffered solution at pH 2.8 (Cooke and Hunt, 1970). This is because of the greater dissolution rate and aqueous solubility of aspirin in alkaline solution and rapid gastric emptying caused by an increase in the pH of the gastric contents. Contrary to the pH-Partition theory, aspirin is absorbed much more slowly from the stomach than from the small intestine (Siurala et al, 1969). The stimulatory effect of alkali on gastric emptying may explain the apparent increase in propantheline absorption caused by sodium bicarbonate (Chaput de Saintonge and Herxheimer, 1973). Alkalis and antacids on the other hand may decrease the rate of absorption of other basic drugs through effect on solubility, and the inhibitory effect of sodium bicarbonate on the absorption of tetracycline may be cited as an example (Barr et al, 1970). Generally antacids alter absorption of drugs by affecting their solubility and dissolution. Tetracycline is poorly absorbed in the presence of aluminum hydroxide, due to chelation (Walsbren and Hueckel, 1950). Sodium bicarbonate, which raises pH without releasing polyvalent cations, also delays tetracycline absorption. For highly soluble and rapidly absorbed drugs such as phenobarbitals (Pka 7.6), sodium sulphadiazine (Pka 6.5), quinine (Pka 8.4) and isoniazid, their passage from the stomach into the intestine is the rate-limiting step in their absorption. Therefore, antacids such as aluminum hydroxides that delay gastric emptying will delay their absorption.

Generally, administering oral medication along with food or at a mealtime is a convenient manner of drug dosing. However, drug interactions can occur that modify the activity of the drug (decrease or increase drug effects) or impair the nutritional benefit of certain food (May, 1997). Examples of drugs whose absorption is decreased when taken with food include Penicillin, Tetracycline, Erythromycin, Levodopa, Phenytoin and Digoxin (May, 1997). Drugs whose absorption increase when taken with food include spironolactone (Melander et al, 1997), griseoflulvin (Crounse, 1961) and itraconazole (Kastrup, 1999). Calcium, Magnesium and aluminium found in food supplements or antacid compounds bind (chelate) with Tetracycline to form an insoluble complex resulting in significantly decreased absorption of Tetracycline (Mc Evoy, 1999). Many drugs, before reaching the systemic circulation are subjected to first pass metabolism by the liver.

Distribution interactions

Theoretically, drug distribution can be affected by numerous factors. Practically all drugs are bound to plasma components, red blood cells and plasma proteins. Protein binding interactions have been studied extensively in – vitro. Concomitantly administered drugs compete for binding sites on blood and tissue proteins to produce displacement interactions. A protein that is very easy to obtain in a relatively pure form and which has been well studied with respect to drug binding is serum albumin. Albumin interacts with a variety of drugs than do other plasma proteins or even many of the intracellular proteins and this provides some clues to the interaction features of proteins. For instance, the albumin molecule which is a single polypeptide chain is spread out. Therefore, the surface area of the molecule is relatively large, which is the reason for the high viscosity of albumin solution. A macromolecule in a single chain

exposes a large proportion of its reactive groups to the reactive groups of drug molecules. Secondly, albumin differs from many other types of protein in that it contains more hydroxy amino acids relative to its carboxy amino acid content, thus leaving numerous unbounded cationic N groups availalable which can then bind anionic drug molecules (Gourley, 1971).

Because only the unbound fraction of the drug is pharmacologically active, an increase in the free concentration of drug increases its pharmacological effects. Many anaesthetics, including volatile anaesthetics, seem to be able to displace drug from plasma protein in-vitro, but this does not appear to have any significant clinical consequences. (Wood, 1991, Grandison et al, 2000). Warfarin, an anticoagulant, is highly bound to plasma protein, when combined with phenylbutazone, warfarin will be displaced from protein binding sites and the concentration of the free warfarin will be increased. The result is enhanced anticoagulant activity with a possibility of haemorrhage.

Binding of drugs to non-receptor site macro-molecules is important because it limits the access of free fraction of drug to receptor sites and to the biotransformation enzymes and excretion process. Binding may be beneficial because it may result in a diminished incidence of side effect and a prolonged duration of action (Brodie, 1965). A number of acidic drugs are attached to only one or two sites on the albumin molecule; for these drugs, the protein has a limited carrying capacity. Many of these acidic drugs appear to compete for the same limited number of non-specific protein binding sites. Hence, competition between co-administered drugs for binding sites in the body can result in displacement of one drug by another, with a resulting rise in the

free and active fraction of a drug. It is widely believed that this displacement or redistribution phenomenon caused enhanced clinical effects and toxicity seen when certain drugs interact in man (Wardell, 1974). Highly bound acidic agents such as phenybutazone, oxyphenbutazone, ethylbiscoumaroacetate dicoumarol, sulfinpyrazole and saliciylic acid are able to displace the long lasting albumin bound sulphonamides from plasma proteins. Since these sulphonamides are not rapidly metabolized or excreted, the displaced molecules diffuse from plasma into tissues with resultant enhanced antibacterial activity. By the same mechanism, pheny butazone increases the antibacterial activity of acidic antibiotics such as penicillin (Hartshorn, 1973).

Drug Metabolism Interaction.

The biotransformation of drugs during the first – pass and during elimination from the systemic circulations in the liver, is usually divided into phase I and phase II reactions. Many drugs are lipophilic and cannot be excreted through the kidneys until they have been transformed into more favourable water soluble forms.

Phase I reactions include oxidation, reduction and hydrolysis. Phase I reactions add a functional group to the drug, where as phase II reaction are conjugation reactions in which the drug or its metabolite is attached to a water – soluble molecule, such as glucuronic acid, glutathione, sulphatic group, acetyl group, methyl group or glucosamine, making the whole complex more hydrophilic. Oxidation is the most important phase I reaction catalysed by cytochrome P450 (CYP450) enzymes. Most metabolic drug interactions involve either the induction or inhibition of cytochrome P450 enzymes (Levy et al, 2000).

Oxidation reactions include aromatic and aliphatic hydroxylation, oxide formation, desulfirization, deamination, dehalogenation, N-O and S dealkylation and

sulfoxidation. Reduction reactions include azoreduction, aldehyde reduction, nitro reduction while hydrolytic reactions include de-esterification and deamination. Oxidative and reducing enzymes are found primarily in the liver microsomes. Hydrolytic enzymes are located in the plasma, liver microsomes and many other tissues.

CYTOCHROME P450 ENZYMES

Cytochrome P450 (Cyp450) enzymes are characterised by a maximum absorption wavelenght of 450nm in the reduced state in the presence of carbon monoxide. According to the homology of their amino acid sequence, the CYP enzymes are divided into families, sub families and specific iso enzymes. CYPI, CYP2 and CYP3 are involved mainly in the metabolism of drugs and other xenobiotics, where as those belonging to the families CYP4, CYP5 and CYP7 have endogenous functions (Levy, 2000).

So many drugs and environmental chemicals are implicated in cytochrome P450 enzymes induction and inhibition. Cytochrome P450 enzyme system inducers include phenobarbital and many other drugs and environmental chemicals, including chlorinated hydrocarbon, insecticides carcinogenic hydrocarbons, food additives and cigarette smoke (Conney and Burns, 1972). Inhibitors of cytochrome P450 enzymes such as phenlybutazone and imidazole compounds have since been recognized (Powell and Donn, 1983). The activities of the cytochrome P450 dependent system are extremely sensitive to difference in sex, age strain and species and to differences in the hormonal and nutritional state of animal (Coney, 1967).

Co-administration of the inhibitor and the substrate of any CYP enzyme will result in an increase of the substrate concentrations. The magnitude of the increase depends on

the inhibitor and its dose. An example is ketoconazole which increased the AUC for oral triazolam approximately 30times compared with the administration of triazolam with placebo (Varhe et al, 1994). But the AUC of oral midazolam was increased approximately 16times (Olkkola et al, 1994).

Many lipid-soluble compounds such as barbiturates phenytoin, carbamazepine and also ethanol cause a stimulation of drug metabolism through the induction of hepatic microsomal enzymes. The administration of the inducing drug causes stimulation not only of its own metabolism, but also the metabolism of many unrelated drugs which are substrates for microsomal enzymes.

Ritonavir is a protease inhibitor used in the treatment of HIV infection. A 2-day ritonavir treatment greatly increases the concentration of intravenous fentanyl by reducing fentanyl clearance. Because fentanyl clearance was reduced by 67%, it can be calculated that ritonavir treatment results in approximately threefold increase in fentanyl concentrations (Olkkola et al, 1999). However, the AUC of norpethidine was increased suggesting the induction of hepatic pethidine metabolism by ritonavir. (Piscitelli et al, 2000). It was shown that paracetamol, a substrate of the CYP2E1 and CYP3A family, does not affect the pharmacokinetics of fentanyl at clinically relevant concentrations (Feierman, 2000).

Ropivacaine is a local anaesthetic, which is metabolised mainly by CYP1A2 but also by CYP3A4. It's clearance is reduced by 77% by concomitant CYP1A2 inhibitor, fluvoxamine. Erythromycine a CYP3A4 inhibitor alone only had a minor effect on the pharmacokinetics of ropivacaine. However, compared with fluvoxamine alone, the combination of fluvoxamine and erythromycine further increased the area under the drug plasma concentration time curve by 50% (Jokinen, 2000).

Recent studies have shown that many dietary supplements and natural products can modify the pharmacokinetics of drug. For instance, St. Johns wort (Hyperuricum perforatum) a plant used as an antidepressant in the United States, is a potent inhibitor of CYP3A4 and can have potentially hazardous drug interactions when used with the substrates for CYP3A4. (Fugh – Berman, 2000).

Drug excretion interaction.

Any interaction here will only be important if a drug or its active metabolite is eliminated principally through the kidney.

Excretion of drug by the kidneys is net effect of 3 processes. Passive glomerualr filtration, active tubular secretion and passive tubular diffusion. Glomerular filtration of a drug is not usually affected by other drugs except in disease states.

However, pH modifies the tubular reabsorption. When the tubular fluid (urine) is alkaline, basic drugs like amphetamine will mostly be in unionised from and will be greatly reabsorbed from the tubular fluid into blood. In this way the action of the drug is prolonged. Acidic drugs (e.g. Nalidixic acid) under the same condition will be mostly be in ionised forms and will not be reabsorbed but excreted. The opposite obtains when the filtrate is acidic. Urine pH effect are best on the elimination of a drug that is filtered at the glomerulus and reabsorbed by non – ionic diffusion back into the blood as the glomerular filtrate passes down the nephron.

Changes in urine pH that increase the ionized fraction of the drug in the urine increase the excretion rate of the drug. This has been extensively investigated with Phenobarbital and alkalinization of the urine (Waddel and Buter, 1957) and put to use treating patients with phenobarbital poisoning (Lassen, 1961).

Some acidic drugs like penicllin, probenicid, phenylbutazone, chlorpropamide are transported from blood into the tubular fluid by an active process which may involve enzyme systems. When two drugs normally excreted by the same active transport mechanisms are given together, one of them will delay the elimination of the other and in this way prolong its action. The prolongation of the action of penicillin by probenicid is well known.

Change in renal function can modify a number of pharmacokinetic processes in the body and thereby lead to unanticipated drug effect or interaction (Rendenberg, 1974). Drug excretion is slowed in patients with impaired renal function

1.1.3 PHARMACODYNAMIC INTERATION

These are drug interactions at the site of action. Many drugs produce their pharmacological effect by combining with specific receptors. Other drugs can occupy these specific receptors without producing any response and in this way prevent or reverse the effect. Example is reversal of the antihypertensive effect of guanethidine by amphetamine. Guainethidine act by blocking nerve transmission on adrenergic neurounes. Amphetamine displaces it from these neurones and thus abolished its action.

Frequently overlooked is the multiplicity of effect of many drugs. Thus, phenothiazines are effective α-adrenergic antagonists; many antihistamines and tricyclic antidepressants, are potent antagonists at muscarinic receptors.

These “minor” actions of drugs may be the cause of drug interactions (Goodman and Gilman, 1996). From the foregoing we see four different kind of pharmacodynamic drug interactions.

(a) Enhanced effects produced by two drugs acting at same site. An example is streptomycine (weak depolarising properties) in the presence of a depolarising muscle relaxant (Toivakka and Hokkanen, 1965).

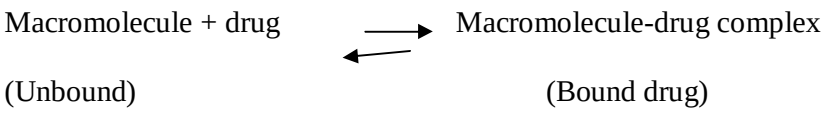
(b) The increased effects produced by two drugs acting at different receptor sites (potentiation). This usually results in an effect, which is greater than the sum of the component effects. E.g Antihypertensive drugs

(c) Enhanced effects of a drug by one which is devoid of action itself. Example of increased anticoagulant effect of warfarin with clofibrate (Solomon and Shrogie, 1967).

(d) Antagonsim of the effect of one drug by another. An example is naloxone action at opioid receptors.

FORCES INVOLVED IN DRUG–MACROMOLECULAR INTERACTION

Generally, the interaction between a therapeutically useful drug and macromolecule is reversible viz:-



This means that covalent bonds, which are very stable at body temperature, are not involved in most interactions between drug and macromolecules in humans. Because of the high bond strengths, covalent bonds are not likely to be broken down, unless specific enzyme is present to break the bond and this may lead to serious drug interactions. However, there are two types of drug – macromolecule interaction that involve the formation of covalent bonds. The first type is the alkylation or arylation of cell constituents by certain drugs that are respectively alkylating or arylating agents.

The second type is the covalent binding of drug or its metabolite to a protein to form a hapten-protein conjugate, called an antigen, which include synthesis of antibodies for the hapten (drug).

The main forces that are involved in the interactions of most drugs with macromolecules in the body are much weaker than the covalent bonds and involve the following;

1. Electrostatic forces:- These forces are formed between ions of opposite charge. This force increase in strength with increasing ionic charge and decreasing distance of approach. Electrostatic bonds are stronger in a medium of low dielectric constant, such as the lipid medium of a membrane, than in a medium of high dielectric constant, such as water. Electrostatic forces play an important role in interaction involving ionizable drugs, and macromolecules having an ionic group such as certain mucopolysaccharides.

2. Hydrogen bonds:- This is a weak or loose bond between the electron deficient hydrogen atoms of hydroxyl, amino and thiol groups and the non-bonding electrons of electronegative atoms such as Oxygen, Nitrogen, Sulphur and Chlorine. Examples are O-HO or N-HO. Usually the atoms are in linear configuration and one hydrogen atom can form only one hydrogen bond and cannot interact simultaneously with a second atom. Hydrogen bond formation may be inter or intra-molecular. Intramolecular hydrogen bonding give rise to ring formation or chelation and this is usually when the formation of a 5,6,0r 7 – membered ring is possible. Hydrogen bonds are weaker than electrostatic forces but stronger than Van der waals forces.

3. Dispersion forces and van der waals forces: Any two electronic systems, for example two atoms or molecules are weakly attracted to each other owing to slight

correlations between the motions of the electrons in the interacting molecules. The attractions arise from natural polarization in electron clouds induced by atoms as they approach one another. The electron clouds are thereby distorted by this polarization and as a result the nuclei of each atom are attracted to the electrons of the other atom. In addition to the attractive interaction of dispersion force, a repulsive force develops between two atoms or molecules when their interatomic distance decreases to the point at which the electron clouds interpenetrate. This combination of attractive and repulsive forces is known as the van der waals force. Altough Van der waals forces are weak and short acting, they are still important element of the binding processes. Hydrophobic interaction describes the van der waals attraction between atoms in the non-polar parts of two molecules immersed in water. Hydrophobic bonding is very important for the binding of small molecules to biological macromolecule.

Hydrophobic interaction are not primarily involved macro-molecular drug interaction but are important for the structure of proteins and nucleic acid and can be disturbed by the approzimation of drug molecules, leading to alteration in the tertiary structure of the macromolecules (Gourley, 1971).

1.2 BASIC CONCEPTS OF PHARMACOKINETICS

When drugs are applied both in-vitro and in-vivo the magnitude of the response is a function of the concentration of the drug in the fluids bathing the sites of action. Therefore to obtain the therapeutic success, an adequate concentration of the drug at the site of action for the duration of therapy is necessary. Pharmacokinetics is the knowledge of the mechanism of drug absorption, distribution and elimination together with the kinetic of these processes. In contrast clinical pharmacokinetics is the application of pharmacokinetic principles to the therapeutic management of patients.

Clinical pharmacokinetics thus attempts to provide both a more quantitative relationship between dose and effect and the framework with which to interpret measurements of concentrations of drugs in biological fluids.

1.2.1 Approach to Pharmacokinetics.

Once a drug is absorbed, it is distributed to the various tissues of the body. The rate and extent of distribution are determined by how well each tissue is perfused with blood, the binding of drug to plasma proteins and tissue components and the permeability of tissue membrane to the drug. Distribution is the process of the reversible transfer of a drug to and from the site of measurement, usually the blood or plasma, where as elimination is the irreversible loss of drug from the site of measurement. Practical clinical pharmacokinetic involves the quantitation of a drug in readity accessible body fluids, followed by attempts to define mathematically the time – course of events.

The attempts to define this time – course of events in the body have produced three philosophical approaches to practical pharmacokinetics:-

(i) Compartment model approach

(ii) Physiological model approach

(ii) Non – compartment approach

The compartment model approach is the conventional method of characterising the pharmacokinetic of a drug. With this approach the body is considered as consisting on one or more compartment with no real anatomical or physiological reality. Another characteristic of this approach in the assumption that rates of drug

absorption, transfer among compartment and drug elimination from compartments all obey first order linear kinetics.

Single – compartment model.

This is the simplest model and depicts the body as a single homogenous unit where the drug entering the body is distributed instantly between the blood and the body fluid or tissues. The exchange of drug between plasma and tissue proceeds more rapidly than the rate of elimination.

Single – compartment open model is actually an approximation or simplification used to describe the two – compartment open model when kab>>kel (Notari; 1975).

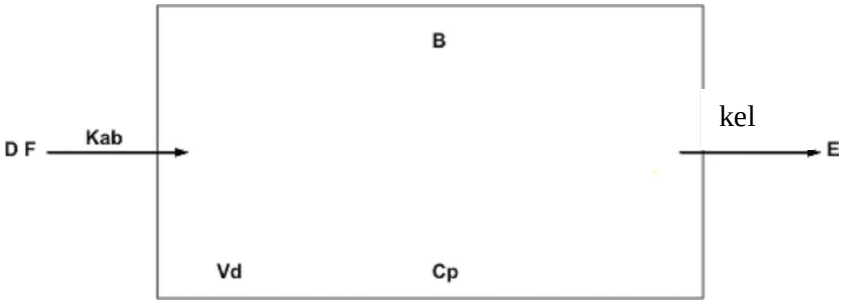


Fig: 1.2.1.1

Characteristics of a single compartment open model

D = Dose of drug administered

F = Bioavailability

Kab = Absorption rate constant

B = Body, (Blood, body fluids, tissue)

Vd = Volume of distribution

Cp = Drug concentration in plasma

Kel = Elimination rate constant

E = Routes of drug elimination

The fact that the body behaves as a one compartment does not mean that the concentration of drug in all body tissues at any given time is the same. However any changes that occur in plasma quantitatively reflect changes in tissue drug levels.

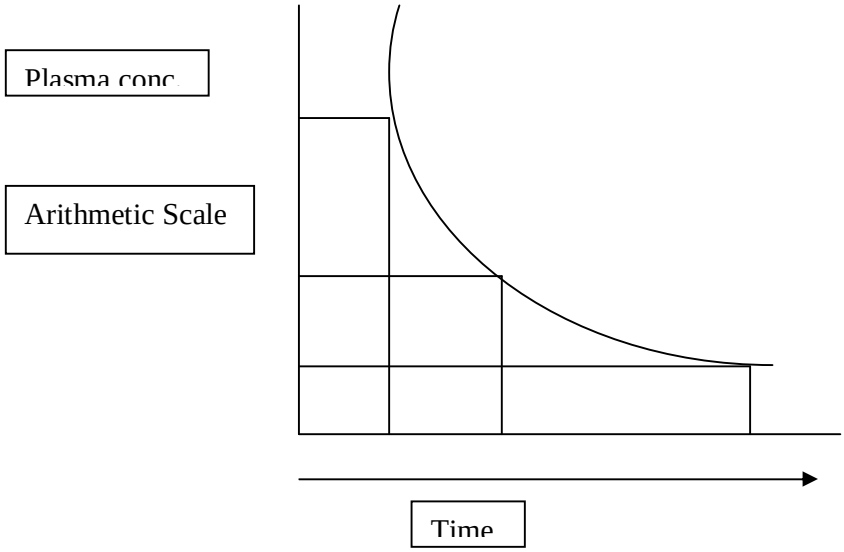
Assuming instantaneous distribution after an intravenous injection of a drug into this model, the concentration (Co) in the plasma immediately after the injection is equal to the dose (D) divided by the volume of the compartment (vd)

$Co = D/v_{d.}$

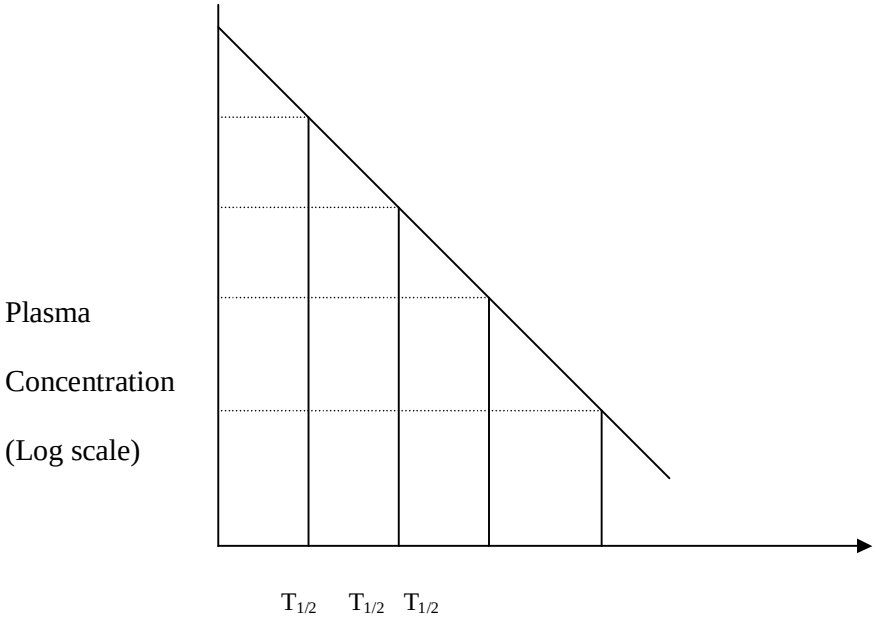
The apparent volume of distribution (vd) is not a true volume, but that volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that in plasma.

Elimination follows a first order kinetics after intravenous distribution. This means that a constant fraction of a drug in eliminated per unit time. A plot of plasma drug concentration against time on the abscissa with each unit representing the time for one half of the drug to be removed, result in the exponential curve shown in **Fig 1.2.1.2**

Re-plotting the curve with concentration on a logarithmic scale, results in a straight line (**Fig 1.2.1.3**). This plot can be extrapolated back to zero time to give Co, the theoretical initial concentration. The elimination half-life (t1/2) of the drug is represented $t^{1/}2$



Plasma fig: 1.2.1.2 Plasma concentration-time plot on a linear (Arithmetic) scale following a rapid IV injection of a drug into a single-compartment open model.



Plasma fig: 1.2.1.3 Plasma concentration-time plot on a logarithmic scale following a rapid IV injection of a drug into a single-compartment open model.

Taking first order kinetics between drug concentration and time, then decline in drug concentration may be expressed as :

$$dc/_{dt} = \beta Ct$$

Where Ct = concentration

β = rate constants of elimination

Integration and conversion to logarithms to the base 10 gives

$$\text{Log } C_t = \text{Log Co} - \beta t/_{2.303}$$

TWO-COMPARTMENT MODEL.

If the distribution of drug is very slow, a two-compartment model must be considered. This model contains a central and a peripheral compartment.

The central compartment, composed of blood and the well perfuse tissues, while the peripheral compartment composed of the tissues or the rest of the body. With this model all drug removed from the body, regardless of the route of elimination is from the central compartment. The central compartment is open since elimination occurs from it. Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissue is rapidly achieved. The combined effect of two compartments gives rise to a biphasic curve on IV injection with two distinct linear portions when drawn on a semi-log scale (fig 1.2.1.4)

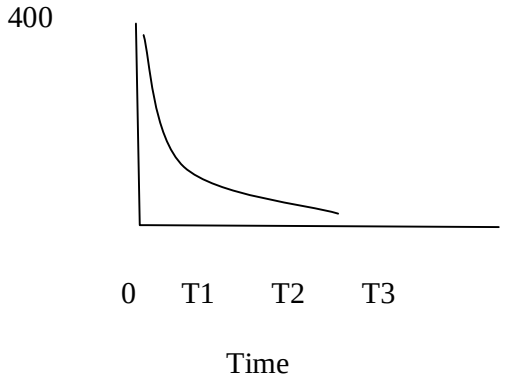
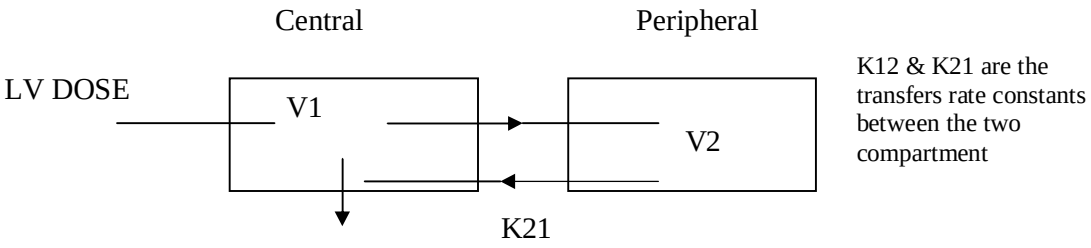


Fig: 1.2.1.4

Even though drug distribution is slow, it is usually faster than elimination. Thus the initial rapid fall in the concentration (distribution phase) mainly represents the relatively rapid process of distribution from central to peripheral compartment. After the distribution, the curve now enters the relatively slow elimination phase (B) during which there is irreversible elimination from the central compartment.



Elimination TWO COMPARTMENT MODEL

Fig: 1.2.1.5

Three - compartment model:

In reality a maximum of three compartments is allowed in assay technique (Paxton.1931). The three – compartment model is a modified model in which an additional compartment in incorporated to represent the volume (vo) from which absorption occurs at a first order rate. (fig 1.2.1.6). It is assumed the entire dose in rapidly introduced into the size of absorption, from which it is absorbed into the central compartment.

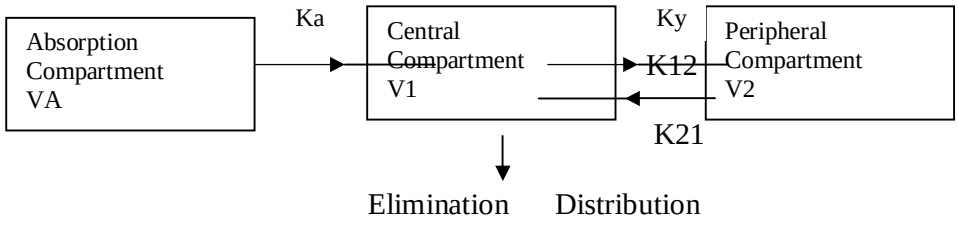


Fig: 1.2.1.6

Ka = Absorption rate constant.

K12 and k21 = Transfer rate constant between the two compartments.

A concentration – time curve for a single oral dose is shown in fig1.2.1.7 This is also obtainable for other administration routes like intramuscular or subcutaneous, which have a preliminary absorption phase.

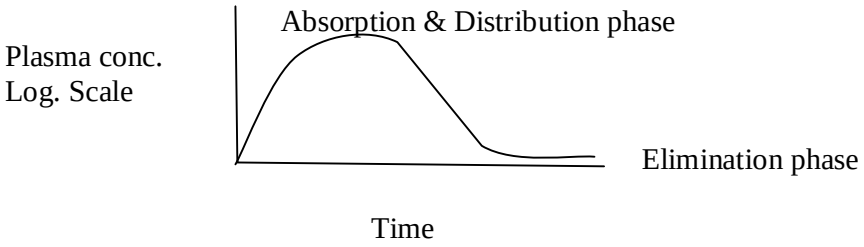


Fig: 1.2.1.7

A conc- time plot after single oral dose.

1.3 PHARMACOKINETIC PARAMETERS

Following drug administration, absorption and disposition are characterised by some important pharmacokinetic parameters. These are; Half-life (absorption/ elimination $t^1/_2$), Area under the curve (AUC), systemic and total body clearance. Volume of distribution (vd), Absorption and elimination rate constants (praxton, 1981). Other are lagtime, Cmax, Tmax.

Clearance

Clearance is the most important concept to be considered when a rational regimen for long-term drug administration is to be designed. (Goodman and Gilman, 1996). It is the volume of plasma from which a drug is totally and irreversibly removed per unit time and is a direct index of drug elimination. The elimination of most drugs follow

first order kinetics. That is, a constant fraction of the drug is eliminated per unit time. If mechanism for elimination becomes saturated, the kinetics becomes zero order. That is a constant amount of drug is eliminated per unit time.

Clearance of a drug is the rate of elimination by all routes normalised to the concentration of drug (c) in some biological fluids

$$CL = \frac{\text{Rate of elimination}}{C}$$

Clearance by means of various organs of elimination is additive.

CL renal + CL hepatic + CL other = CL systemic

Meaning Total systemic clearance is the sum of hepatic, renal and other clearance like lungs.

Area under the curve (AUC).

This is the area under the concentration-time curve from time zero to infinity (AUC) and is a measure of the extent of drug absorbed into the systemic circulation (Bioavailability). Some of the ways AUC may be obtained are;

1. Use of a planimetre
2. Cut and weigh method
3. Trapezoidal triangular method.

In the trapezoidal method, AUC is estimated by dividing the curve into sections that approximate a series of trapezoids with a triangle at each end. The individual area of the trapezoids and triangles are summed to obtain the AUC (Notari, 1975)

Apparent Volume of distribution (vd)

This is the volume in which the amount of drug in the body would need to be uniformly distributed to produce the observed plasma concentration. This volume does not necessarily refer to an identifiable physiological volume, but merely to the fluid volume that would be required to contain all of the drug in the body at the same concentration as in the blood or plasma.

$$V = \frac{\text{Amount of drug in body}}{C}$$

Vd can be determined from the relationship.

$$Vd = \frac{F \times D}{AUC}$$

F = Bioavailability

D = Dose

AUC = Area under the curve

As might be expected, the volume of distribution for a given drug can change as a function of the patient's age, gender, disease and body composition (Goodman and Gilman, 1996).

Elimination Half – Life ($t^1/_2\beta$)

This is the time taken for half the Amount of the drug present in the body to be eliminated either by extraction or metabolism or both.It is infact the time taken for the plasma concentration to reduce by fifty percent of its original value (Wagner, 1986) and can be determined for either one or two compartment model.

1.4 Spectroscopy

Spectroscopy is the study of interactions of electro-magnetic radiation with matter. Such interactions of energy with organic molecules lead to changes in either electronic, vibrational or rotational energy inherent in the molecules all of which constitute what is known as the internal energy of a molecules. A plot of percentage transmittance (or of absorbance) against wavelengths gives a picture described as a spectrum. Various regions of the electromagnetic spectrum have applications in pharmaceutical sciences in general. In particular, the techniques frequently employed in pharmaceutical analysis include ultra-violet (colorimetry), infrared and atomic absorption and emission spectroscopy. The spectral range for these measurements can for convenience be divided into ultra violet (190 to 380nm), the visible (380 to 780nm) the near infra red (780-3000nm) and the infra red (2.5 to 40mcm or 4000 to 250 /cm).

1.4.1 Ultra-viloet/visible absorption spectrophotometry

The commonest physico-chemical method and with the most application in analysis of drugs in Biological sample is uv/visible spectrometry. This provide characteristic spectrum that provide information on the identity or structure of the analyte, thus useful for qualitative analysis and give quantitative information as well. The quantitative information depend on the extent of absorption. This can be described in terms of Beer-lambert law; Lamberts law relates the total absorption to the optical pathlength:

Absorption(A)= log10 (I/I) = kl

I= incident light intensity, I= transmitted light

L=path lengths

K=proportionality constant for material

Beer law relates absorption to the concentration of the absorption solute, c, in solution.

Log10 (I/I) =kc (the path length l, being constant)

Combining the two equation you get

Log10 = ecl

E=constant for a particular solvent at a particular wavelength

With the advent of continous scanning spectrophotometer, with the control of the spectrphotometric functions (wavelengths, filter, radiation source etc.) are incorporated with computer. The characteristic single spectrum, which gives a sharp peak, is achieved at a particular wavelength called maxima (max). But compounds with the same functional group will have a similar absorption spectra, the technique is therefore not suitable for identifying unknown components in a sample. The technique is also not suitable for quantitative analysis if metabolites with same functional groups are present. The use of appropriate solvent at a particular PH ensures only parent drug is extracted and this further ensures specificity of absorption at a particular wavelength. If a specific extraction step is applied hence absorption methods are only specific for unmetabolised drug, as most drug metabolites are more polar than the parent drugs (Chamberland 1995)

Ultraviolet-visible spectrophotometry is best known as a tool for quantitative analysis. Speed, simplicity and sensitivity have made the UV-visible spectrophotometry a popular tool among analysts for the quantification of drugs and metabolites in biologic samples. One of the main reasons for its popularity is that the sensitivity of the method is in the range of 1-10 µg/ml, which is comparable to the concentration

level of many drug substances in biologic samples (Smith and Stewards, 1981). The following are some applications of UV spectrophotometry in pharmaceutical analysis. Mustapha et al (1996) reported a UV method to study the effect of Tamarindus indica on the bioavailability of aspirin in healthy human volunteers. In this method, plasma samples (1ml) were placed in a 20ml extraction tube and 2ml of 0.05M Hcl was added. 10ml of ethylacetate was then added and the mixture shaken for 15min at 3000 rpm. The supernatant was then transferred into a second extraction tube and evaporated to dryness on a water bath maintained at 40 +_10°C under a gentle stream of nitrogen passing through the tube. The residue was reconstituted in 5ml methanol and absorbance taken at 231nm and 276nm respectively. Concentrations of aspirin and its metabolite, salicylic acid in the plasma were estimated using the simultaneous equation for multiple component mixture.

Garba et al (1999) reported a UV method to study the influence of Cimetidine on the pharmacokinetics of paracetamol in healthy subjects. According to the method, saliva samples (2ml) were placed in 10ml extraction (centrifuge) tube using auto-pipette. Ethyl acetate (5ml) were added to the content of the tubes and well stoppered. The mixture was vortex mixed for one minute and centrifuged at 2500rpm for 5minutes. The ethyl acetate layer (upper) was removed with pasture pipette and its absorbance measured at 262nm, using 1cm Silica cuvettes using a double beam SP8-100 UV Spectrophotometer.

Drugs can be determined either as intact compound or as a derivative formed by chemical reaction with suitable reagents (Smith and Steward, 1981) the advantage of chemical derivatization as an aid to UV analysis is that the sensitivity of the UV procedure is increased significantly. Example, it has been reported that the sensitivity

of the UV procedures for amitryptylline was increased significantly by oxidation of the drug to anthraquinone. Anthraquinone shows stronger absorption around 250nm than the drug itself (Hamilton et al, 1975)

In a study to determine paracetamol in the urine. The UV spectra of investigated samples were recorded over the wavelengths range 220-400nm (step 0.21nm,scan speed 60nm/min) and second order derivative spectra were calculated. Second order derivative spectra of different blank urine samples displayed the presence of a zero-crossing point at 245-247nm defined as zc. The zero order absorption of paracetamol in water displays maximum absorption at 243nm, while in second derivative spectra, a minimum peak at 246nm was observed. Therefore, the application of zero – crossing technique to the second derivative UV absorption spectrum should be useful for the determination of paracetamol using Dzc. The obtained results were in good accordance with published data on cumulative urinary excretion after per oral administration of paracetamol obtained applying different spectrophotometric methods of determination (Jelena et al, 2003).

Another type of chemical derivatization method uses conversion of a drug to a stronger UV absorbing species by an acid catalyzed re-arrangement (Fellenberg and Pollard, 1976). In the procedure carbamazepine is extracted from blood, with dichloromethane, the solvent is then evaporated to dryness and the residue treated with HCl at 150^0C. After extraction of the acidic solution with n-heptane, its absorbance is measured as 258nm.

In the UV assay of methadone, the compound is subjected to an alkaline extraction into n-hexane and back extraction into a ceric acid-H^2SO4 solution. After refluxing, the generated benzophenone is measured in the n-heptane layer by UV

spectrophotometer. Amount of methadone as low as 5 µg can be detected in biological specimens.

Second-derivative UV spectrophotometric method for the determination of naproxen in the presence of its metabolite, 6-0- desmethylnaproxen was reported (Panderi and Parissi, 1994). According to the procedure, plasma (1ml) was acidified with 0.5ml of 1M HCl and 5ml of diethyl ether were added. The mixture was vortex mixed for one minute and centrifuged at 4000 revolution per minute for 10minutes. After refrigeration at -17^0C, the organic layer was placed in a glass vial and evaporated to dryness at 37^0C, under a stream of nitrogen. The residue was dissolved in 2ml of 0.1M–NaOH and the second derivative UV spectrum of the solution was recorded from 280 to 350nm and the amplitude at 328.2nm was measured. The calibration graph was linear from 5-100mg/L of naproxen, with a determination limit of 2.42mg/L. The result obtained agreed with those obtained by HPLC (Panderi and Parissi, 1994)

1.4.2 ANALYTICAL METHOD DEVELOPMENT

One of the first practical steps in the development of a method is to examine and know the chemical characteristics of the drugs you want to analyze. Structural information on the drug including NMR, and MASS-spectra are very vital. Information on the UV-spectra are also very important both for qualitative and quantitative applications, like in chromatography.

Another very important consideration, which may well dictate a method to be adopted is the sensitivity required, that is the lowest concentration which could be measured after optimization of analytical conditions. Concentration range for which various

technique are applicable has been developed by (De Silva, 1995). A diagram showing sensitivity of the difference technology is presented in **Fig 1.4.2.1**

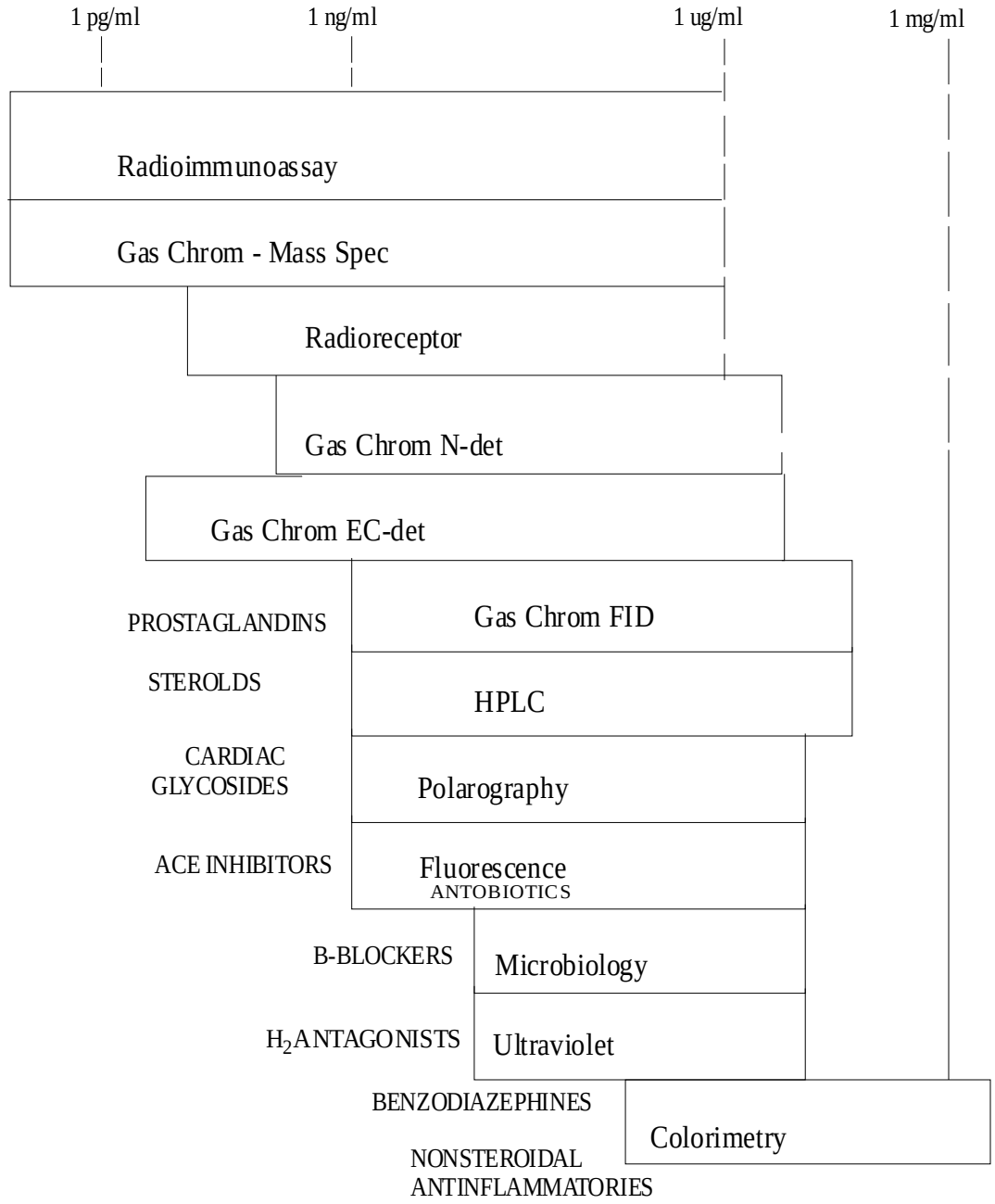


Fig: 1.4.2.1

Diagramme of concentration range for which various techniques are applicable.

This diagram helps in the selection of the technique available for development of analytical method within sensitivity range. There is a considerable improvement in the sensitivities of the analytical methods over time. From mg/ml in the 1950s to femtogrames per ml being claimed in the 1990s.

The most sensitive detector yet developed appears to be the laser induced fluorescence detector, which in conjunction with capillary electrophoresis chromatography can detect 10 Yoctomytes of analyte, or sire molecules (Chambland, 1995).

VALIDATION OF ANALYTIC METHOD

Analytical method validation includes all the procedures followed to ensure that a particular method for the quantitative determination of an analyte or series of analytes in biological material is reliable for the intended application.

The objectives of analytical method validation include the following:

1. To build confidence in analytical data generated
2. To ensure that a selected analytical procedure will give reproducible and reliable result. It is therefore, necessary to validate a method according to the well established criteria of precision, accuracy, sensitivity, specificity and reproducibility.

PRECISION

The precision of an analytical method is the degree of agreement among individual tests when the procedure is applied repeatedly. Precision is usually expressed as standard deviation (Coefficient of variation) C.V. Where a small standard deviation indicate higher precision.

Precision in usually determined by assaying sufficient number of aliquots of a homogenous sample in order to be able to calculate statistically valid estimate of mean (x), standard deviation (SD) or the coefficient of variation (CV). Both within-day and between-day precisions are relevant in the development of analytical method.

PERCENTAGE EXTRACTION RECOVERY

The percentage extraction recoveries of analytical method give the efficiency of the extraction procedure to be employed in the analyses. It also gives assurance on the reproducibility of the extraction method employed.

The percentage extraction recovery is calculated with the following equation.

$$\frac{\text{Amount in conc. recovered after sample extraction}}{\text{Amount in conc. Recovered after extraction in O.1N Hcl}} \text{ X } \frac{100}{}$$

CHAPTER TWO

LITERATURE REVIEW

2.1 PARACETAMOL

Paracetamol is the most commonly used analgesic-antipyretic pharmaceutical and it has been in common use in the united state and throughout the world for many years (Dwight, 2000). Paracetamol (acetaminophen) was first used in medicine by Von mering in 1893. However it has gained popularity only since 1949, after it was recognized as the major active metabolite of acetanilide and phenacetin (Goodman & Gilman 1996). Acetanilide and phenacetin were used previously between 1886 and 1887 but were found to be very toxic. The complete prohibition of the use of phenacetin as an active ingredient of medicines in 1979 has made paracetamol to become the most widely accepted alternative to aspirin. The relatively recent clinical recognition of the association between Reye's syndrome and salicylic use in the 20^{th} century also led to the clinical acceptance of paracetamol as the primary pharmaceutical of choice for the control of fever and pain. Also, unlike aspirin it is well tolerated by ulcer patients.

2.1.1 CHEMISTRY

Paracetamol is 4-acetamidophenol and may be represented by the following formula

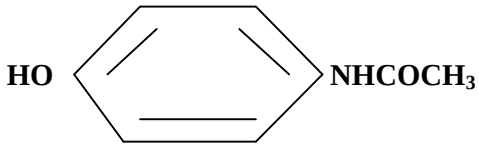


Table2.1.1.1 Physicochemical characteristics of paracetamol.

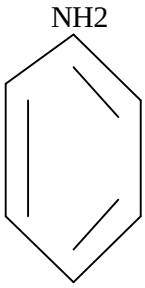
1. Molecular formula $C_8 H_9 NO_2$
2. Description: White, odorless crystalline powder with a bitter taste
3. Melting point: 169 -172^{0}C
4. Pka: 9.5 (weak acid)
5. Molecular weight: 151.2
6. PH: 6 (a standard aqueous solution)

In the US pharmacopoeia it is known as acetaminophen. Paracetamol is a white, odorless, crystalline powder with a bitter taste, soluble in 70 parts of water (I in 20 boiling water), 7 part of alcohol (95%) 13 part of acetone, 40 part of glycerol, 9 part propylene glycol, 50 parts of chloroform, or 10 part of methyl alcohol. It is insoluble in benzene and ether. A saturated aqueous solution has a pH of about 6 and is stable (half life over 20 year) but stability decreases in acid or alkaline condition, the paracetamol being slowly broken down into acetic acid and P-amino phenol. (Fairbrother J.E, 1974) it is also soluble in ethyl acetate and dimethylformamide (The pharmaceutical codex, 1994). Mixtures of paracetamol and aspirin are stable in dry conditions, but tablets containing these two ingredients, particularly in the presence of moisture, magnesium stearate or codeine produce some diacetyl-p-aminophemol when stored at room temperature and this latter compound is hydrolyzed in the presence of moisture to paracetamol and p-aminophenol. The degradation of paracetamol is both acid catalysed and base catalysed and is first order with respect to paracetamol, hydrogen ion and hydroxide ion concentration. The degradation product in addition to p-aminophenol, produces acetic acid. P-aminophenol may degrade to

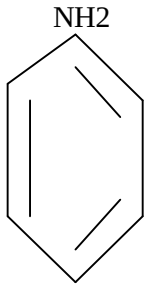
quinomine with resultant change in colour. Paracetamol therefore requires protection from light, temperature and humidity (The pharmaceutical codex, 1994).

2.1.2 STRUCTURE – ACTIVITY RELATIONSHIP

The antipyretic activity of the compound resides in the aminobenzene structure. The introduction of other radicals into the hydroxyl group of paracetamol and into the free amino group of aniline reduces toxicity without loss of antipyretic action.

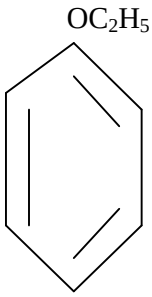


Aniline



Para Aminophenol

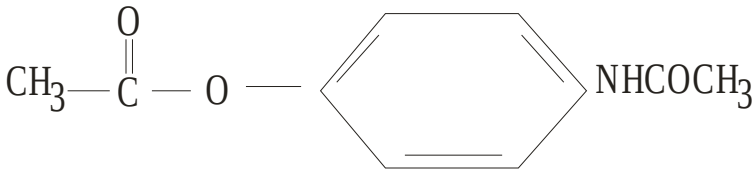
Best results are obtained with phenolic alkyl ethers (phenacetin) and with the amides (eg Acetaminophen, phenacetin) (Goodman & Gilman 1996).



Phanacetin

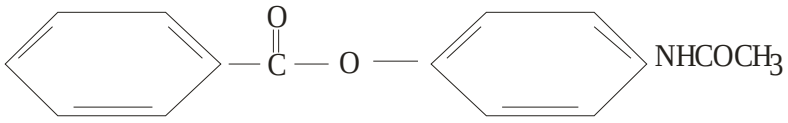
Esterification of the OH-group with an acetyl moiety produced analgesic that has the same activity and disadvantages as the free Phenol e.g:

P-acetoxyacetanilide



P-acetoxyacetanilide

The salicyl ester exhibit diminished toxicity and an increased antipyretic activity.



Salicylester

(Phenestal

2.1.3 PHARMACOKINETICS

ABSORPTION:

Paracetamol is readily absorbed from the gastointestinal tract with peak plasma concentrations occurring about 10 to 60 minutes after oral administration. Paracetamol is a weak acid with pka of 9.5. It is therefore, largely unionized in both the stomach and the small intestine and should be well absorbed from both sites (Schanker et al, 1957).

Generally drugs are absorbed better in the small intestine and a good correlation has been found between stomach emptying and peak plasma concentration for paracetamol (Heading et al 1973).

Also drugs like propantheline (Nimmo et al, 1973), Pethidine,

diamorphine (Nimmo et al, 1975) and desmethylimipramine (Hall et al 1976) which delay gastric emptying, also delay the absorption of Paracetamol.

Neonatal gastric pH is almost neutral and although this favours absorption of paracetamol the stomach is not an optimal entry point to the circulation. Paracetamol therefore is ideally absorbed from the small intestine. The large surface area, ideal blood flow and permeability favour absorption of the majority of drugs. Absorption from this area however depends on gastric emptying which is slow and erratic in a neonate. Slow absorption of paracetamol in infants less than three months old has been demonstrated (Anderson et al, 2000). This shows how age affects paracetamol absorption.

The pharmacokinetic of paracetamol were also dose dependent during a single dose bioavailability study. The type of formulation also affects the availability of paracetamol preparations. It was found that the absolute availability of an elixir was significantly greater than of tablets (87% and 79% responsively). (The pharmaceutical codex, 1994).

The intra individual variation in the absorption of paracetamol though present is insignificant after a semi solid meal. As shown in a study the intraindividual variability was low with coefficients of variation 38. 3%, 8. 0% and 3.8% for time to maximum plasma concentrations, maximum concentration and area under the plasma time curve until 6 hour respectively. (Pintaud et al 1998). Heading et al, (1973) also observed some inter individual variability in the absorption of Paracetamol, they found that peak Plasma concentrations after oral administration of 1.5g Paracetamol to 14 patients varied from 7.4 to 37.0 µg/ml and the time taken to reach these levels ranged from 30 to 180 minutes. They suggested that this variation might be due to individual differences in the rate of gastric emptying.

The presence of certain foods in the GIT influence gastric emptying and consequently absorption of paracetamol for example fatty and carbohydrate food, decrease gastric empting. The presence of certain drugs like the Anticholinergics example atropine, narcotics like morphine and analgesics like Aspirin, all decrease gastric emptying (Meyersohn, 1971) effervescent tablets are absorbed significantly faster than ordinary paracetamol tablets (Rygnestad et al, 2000).

In a study it was found that paracetamol absorption can be used as a marker of gastric emptying due to good correlation between scintigraphy and the paracetamol absorption technique. (Willems et al, 2001). Absorption of paracetamol following rectal administration of panadol suppositories to post operative children is slower and reduced as compared to oral therapy (Coult hard et al, 1998). Also following rectal administration in children, pharmacokinetics models suggest that absorption of acetaminophen is a function of zero order dissolution of suppositories and first order absorption from the rectum. Suppository dose size also affect absorption characteristics and the acetaminophen dose of 10-15mg/kg yields peak serum concentrations less than the antipyretic serum concerntrations of 10-20micro/ms. The initial does should therefore be 40mg/kg (Birmingham et al, 1997).

DISTRIBUTION:

Paracetamol is relatively uniformly distributed throughout most body fluids (Goodman & Gilman, 1996). In humans, usual analgesic doses produce total serum concentration of 5 to 20 µg/ml. A good correlation between serum concentration and analgesic effect has not been reported (Drug fact and comparison, 1989)

Paracetamol exhibit negligible protein binding (Gazzard et al, 1973) and has a high lipid solubility. However, 15-21% is protein bound at concentration of 280µg/ml,

corresponding to the levels observed after human overdose (Gazzard et al, 1973). In neonates, a slightly higher proportion of the drug is bound to plasma proteins compared with adults. The sulphate and glucuronide conjugates of paracetamol do not bind to plasma proteins even in the relatively high concentration found in anaphoric patients (Lowenthal et al, 1976).

A linear process for its transport into the brain based on passive diffusion is postulated (Gazzard et al, 1973, VanBree et al, 1998). It has a CSF to plasma partition coefficient of 1.18 (Anderson et al, 1998). In comparison to atenolol, paracetamol being more lipophilic achieved higher concentration in the brain (de Lange et al, 1998)

The elimination half-life varies from 1-3 hours. Paracetamol has a large volume of distribution. As a result irrespective of whether treatment is being given rectally or orally it is only possible to achieve an effective therapeutic blood level quickly if the first dose is given in large “loading dose”. An effective blood level will only be achieved after three or four doses have been given if a loading does is not given. This is why 30mg/kg, a dose twice the normal recommended dosage is needed to bring fever under control (Treluyer et al, 2001).

It crosses the placenta and is present in breast milk. Breast milk and plasma levels of paracetamol were monitored in 3 lactating women after ingestion of a single 500mg dose of paracetamol. The paracetamol concentrations were consistently lower in milk with a mean milk/plasma AUC ratio of 0.76. This value was in close agreement with milk/plasma partition of 0.81 found in vitro, and could be related to quantitative binding differences between the two fluids. The half-lives of paracetamol in plasma and breast milk were almost identical with an overall mean of 2.7 hour. As less than

0.1% of the maternal dose would be present in 100ml milk, breast-feeding need not be discontinued due to paracetamol treatment in the conventional dosage (Bitzen et al, 1981).

In another study the absorption parameter from saliva (cmax, tmax, AUC and saliva levels) correlated well (r=0.88 to 0.99) with those from plasma. The plasma levels were higher than saliva in all subject studied (Chinedum et al) . Paracetamol has been found in human Saliva in concentrations which correlate well with plasma levels (Glynn and Bastain, 1973)

No marked localization of the drug in any organ has been reported except in the pituitary. In contrast to the salicylates, however, a considerably greater portion of acetaminophen enters the brain and presumably other tissues, and much less remains in the blood. In animals the concentration of drug in the brain is equal to that in the blood 30 minutes after administration. It disappears more rapidly from the brain than does aspirin, perhaps resulting in briefer analgesia and antipyretic action than aspirin (Davison et al, 1961).

METABOLISM

Paracetamol is primarily metabolized by the liver. Most of it is combined with glucuronide and sulphate. After therapeutic does, 90% to 100% of the drug may be recovered in the urine within the first day, primarily after hepatic conjugation with glucuronic acid (about 60%), sulfuric acid (about 35%): small amounts of hydroxylated and deacetylated metabolites have also been detected (Goodman and Gilman, 1996). Less than 5% of paracetamol is excreted unchanged.

The manner in which the liver metabolises paracetamol changes with age. There is a reversal of the usual adult ratio of 2:1 with respect to glucuronidation vs sulphation of

paracetamol in young children. This pattern reverts to adult pattern at the age of 12 years (Miller et al, 1993).

A small proportion of paracetamol undergoes cytochrome p.450 – mediated N-hydroxylation to form N-acetyl-benzoquinoneimine, a highly reactive intermediate. This metabolite normally reacts with sulphydryl groups in glutathione. As the dose of paracetamol increase the quantity of benzoquinoneimine increased too. There then comes a point where the glutathione stores in the liver have been completely used up, under these circumstances, reaction with sulphydryl groups in hepatic proteins is increased and hepatic necrosis can result. In newborn infant glucuronide formation of paracetamol may be delayed because the enzymes synthesizing systems are not completely developed, perhaps leading to enhanced toxicity (Vest and Streiff, 1959)

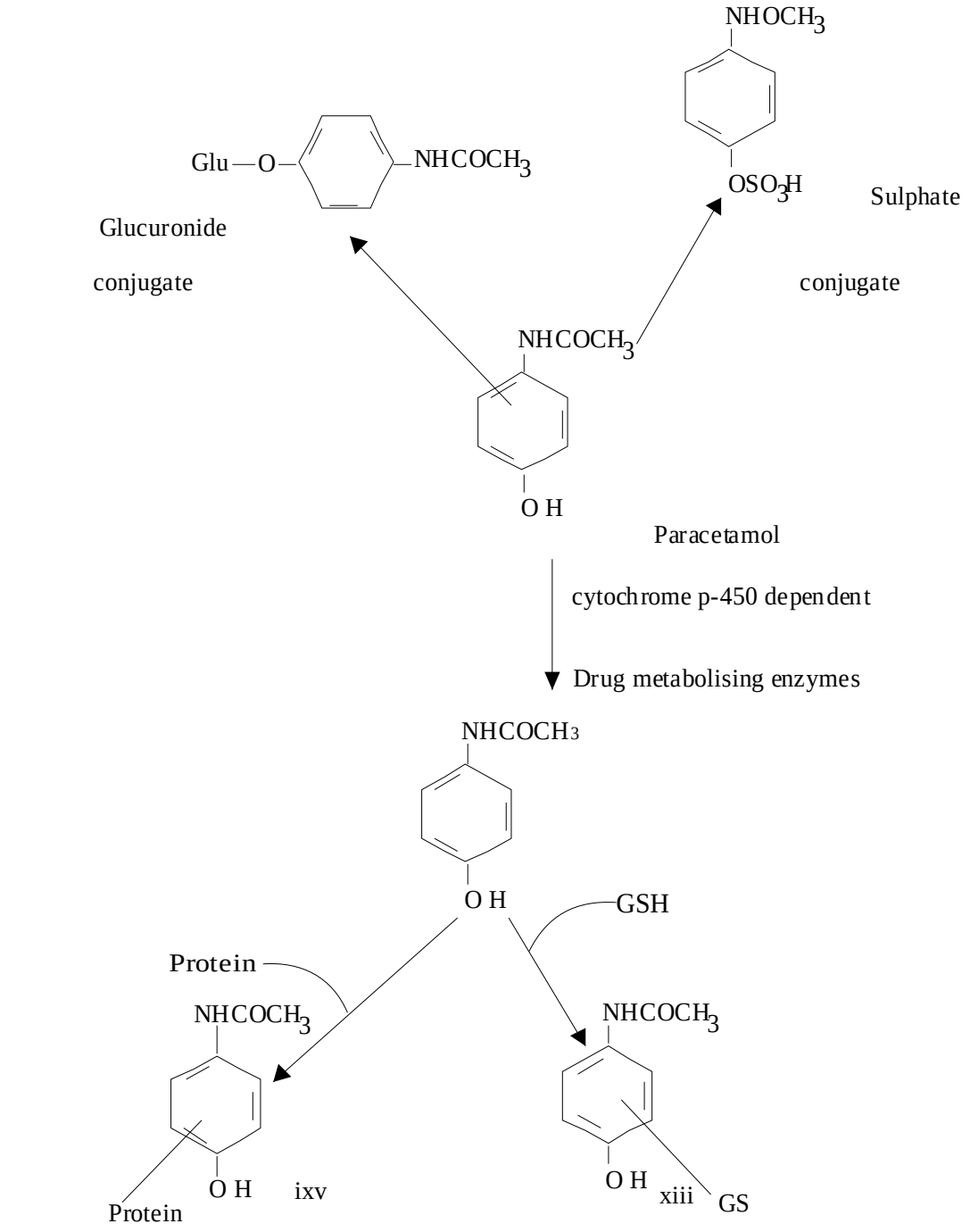


FIGURE 2.1.3.1 Pathway of paracetamol metabolism (GSH, glutathione; GS, glutathione, moiety, GLU, glucuronide moiety. Adapted from Correia, (1995)

In a study it was found that food and water deprivation in calves impairs the formation of major metabolites (glucuronide and sulphate) of paracetamol (Janus et al, 2003). Toxic doses of paracetamol result in impaired energy coupling in the liver mitochodria in the rat. Effects of subtoxic doses were also demonstrable in terms of impaired dehydrogenases activities.

TOXICITY

In recommended does, aceminophen is usually well tolerated. Skin rash and other allergic reactions occur occasionally. The rash is usually erythematous or urticarital but sometimes it is more serious and may be accompanied by drug fever and mucosal lesions. In a few isolated cases, the use of paracetamol has been associated with neutropenia, thrombocytopenia and pancytopenia. (Goodman and Gilman, 1996). The most serious adverse effect of acute overdosage of acetaminophen is a dose-dependent, potentially fatal hepatic necrosis (Thomas, 1993). In adults hepatotoxicity may occur after ingestion of a single dose of 10 to 15g (150 to 250mg/kg) of paracetamol.

As shown under metabolism the hydroxylation metabolite of paracetamol, N-acetyl benzoquinoneimine reacts with sulfhydryl group in glutathione stores in the liver. Following over dosage, the metabolite is formed in amounts sufficient to deplete hepatic glutathione, under these circumstances, hepatic necrosis result, perhaps in part as a result of intracellular accumulcation of ca $^{2+}$, activation of ca^{2+} – dependent endo nuclease and resultant DNA fragmentation. (Goodman and Gilman, 1996) N-acetylbenzoquinoneimine is highly protein reactive (electrophilic and arylating). It damages key intracellular proteins primarily by arylation of protein, a process that involves a series of reactions and in some circumstances by covalent binding. The

process may be reversible at variable points in time when varying degree of cell injury have progressed and therefore may not involve irreversible covalent binding of hepatic macromolecular protein (Dwight, 2000). Symptoms that occur during the first 2 days of acute poisoning by acetaminophen may not reflect the potential seriousness of the intoxication. Vomiting, anorexia and abdominal pain occur during the initial hours and may persist for a week or more.

Paracetamol (5mmol/kg i.p) caused liver damage in rats as indicated by increased plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutamate dehydrogenase (GDH) activities. No change in bilirubin or creatinine was noted (Flutter et al, 2001).

MANAGEMENT OF PARACETAMOL POISONING

Treatment of overdose consists of skilled and prompt hospital management of the patient. Gastric lavage should be carried out especially if the overdose was taken in the last 4 hours. All supportive measures should be instituted. Fluid and electrolyte therapy is instituted in the early stages if nausea and vomiting presented. Intravenous sodium bicarbonate is often given but there is no evidence that this affects the out come (Graf and Ariett. 1986).

Activated charcoal is given to reduce gastrointestinal absorption, especially in multiple drug overdosage. Antidote therapy should be started as soon as possible after suspected paracetamol ingestion. Acetylcysteine is the most effective antidote of choice. It is preferably given via intravenous route although may be given per oral. Acetycysteine is most effective when administered during the first 8 hours following ingestion and the effect diminishes progressively between 8 and 16 hours. Starting treatment after 15 hours was considered of no benefit. However late administration

has now been shown to be safe (Parker et al, 1990) and beneficial result may be obtained beyond 15 hours.

Methiominine is an alternative to acetylcysteine and is most effective when given as early as possible. However, it is not as effective in late presentation. (Janes et al, 1992) (Vale et al, 1981)

Liver transplantation has been considered as a last recourse in some patients.

EXCRETION:

The elimination half- life of paracetamol varies from about 1 to 3 hours. Paracetamol is excreted in the urine, mainly as the glucuronide and sulphate conjugates. Less than 5% is excreted as the unchanged paracetamol (Martindale, 1996). The elimination half-life may be extended from 4 to 8hrs with toxic doses (Rang and Dale, 1991).

The renal tubular transport of paracetamol and its conjugates was investigated with renal clearance and stop flow studies in the dog. Paracetamol is sparingly bound to plasma proteins and therefore undergoes glomerular filtration. It is reabsorbed in the renal tubules by simple diffusion. The conjugates of paracetamol, the sulphate and the glucuronide, both undergo glomerular filtration being weakly protein bound. At low concentration in plasma both are secreted by an active transport process. At higher concentrations both compounds are reabsorbed. Clearances are not dependent on urinary pH or flow rate. It is concluded that reabsorption is not a passive process but that there is an active bidirectional transport of the conjugates. Net tubular secretion of the sulphate, but not the glucuronide conjugates was inhibited by the administration of probenicid (Duggin and Mudge, 1975). The absorption and elimination of paracetamol were unaffected by renal impairment. However the area under the plasma concentration time curve and the elimination half life of paracetamol metabolites

increased significantly with worsening renal insufficiency. Mean renal clearances of paracetamol and its conjugates were significantly reduced in these subjects. There was no evidence of altered metabolic activation with renal impairment. These demonstrate that paracetamol disposition is minimally affected by diabetic nephropathy. However, extensive accumulation of conjugates may occur (Chan et al 1997). It has been suggested that paracetamol itself may be regenerated from these metabolites (Prescott et al, 1989; Martin et al, 1991).

2.1.4 PHARMACODYNAMICS

Paracetamol has analgesic and antipyretic effects that do not differ significantly from those of aspirin. However it has only weak anti-inflammatory effect (Goodman and Gilman, 1996) Paracetamol is almost as potent as aspirin in inhibiting prostaglandin synthetase in the central nervous system (CNS), but its peripheral inhibition of prostaglandin synthesis (anti-inflammatory) is minimal.

The weak anti inflammatory action of paracetamol is because it is a weak inhibitor of cyclo oxygenase in the presence of the high concentrations of peroxides that are found in inflammatory lesions (Marshall et al 1987, Hanel and Lands, 1982). Paracetamol does not inhibit neutrophil activation as do other NSAIDS (Abramson and Weissmann, 1983) Paracetamol has a central action for antipyresis in the hypothalamus (Van Arman et al, 1985; Flower and Vane, 1972) and is believed to exert its analgesic effect by acting on receptors involving N-Methyl-d-aspartate (NMDA) and substance P in the spinal cord (Pilleta et al 1991) (Bjorkman et al, 1994).

Now recent research has shown the presence of a new, previously unknown cyclo oxygenase enzyme, COX-3, found in the brain and spinal cord, which is selectively

inhibited by paracetamol, and is distinct from the two already known enzymes cyclooxygenase enzymes COX-1 and COX-2. It is now believed that this selective inhibition of the enzyme COX-3 in the brain and spinal cord explain the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted GI side effects (Chandrasekharan et al, 2002).

Paracetamol is equivalent to aspirin as an effective analgesic and antipyretic drug. But it is not a good substitute to aspirin as an anti inflammatory drug. It is useful in mild to moderate pain, headache, post-partum pain, myalgia, anti pyresis in certain bacterial and viral infections.

It is a good substitute to aspirin in patients that are allergic to aspirin. It is also preferable to aspirin in patients with haemostatic disturbances, bleeding diatheses (eg. Heamophilia), peptic ulcer disease and in those with bronchospasm precipitated by aspirin.

2.1.5 DOSAGE

Dose: By Mouth, 0.5-1g every 4 -6 hours to a maximum of 4g daily.

Child: 2 months 60mg for post – immunization pyrexia.

3 months to one year 60 – 120mg, 1 – 5 years 120 – 250mg, 6 – 12 years 250 – 500mg: these doses may be repeated every 4 -6 hours when necessary (max. of 4 doses in 24 hours) (BNF, March 2005).

2.1.6 SALIVA CONCENTRATION OF PARACETAMOL AND ASSAY METHODS

Drug levels in saliva can be used to estimate absorption pattern of drugs. In a study, the absorption parameters of paracetamol from saliva (Cmax, Tmax. AUC and saliva

levels) correlated well (r = 0.88 to 0.99) with those from saliva, even though the plasma levels were higher than the saliva plasma in all subject studied (Chinedum et al). This study shows that saliva levels of drugs can be used to determine absorption kinetics of drugs. A recent review has shown that for many drugs there is a high degree of correlation between the saliva and plasma concentration of drug (Danhof and Breimer, 1978). This is being used to advantage because collection of and measurement of drug concentration in the Saliva presents a non-invasive method of obtaining information about plasma drug concentration. Acetaminophen is well secreted into saliva. There is an apparent good correlation between saliva and plasma concentration (Glynn and Bastain, 1973). The concentration of acetaminophen in saliva is high enough to obviate the need for highly sensitive analytical procedures, furthermore, the half life of acetaminophen is short enough so that a fairly good characterization of the time course of the drug in the body may be obtained in three hours.

The measurement of the salivary concentration is of most value in studies on compounds that are less ionized at physiological pH values (Graham, 1982).

In a more recent study, Cone (1993) has shown that there was a high correlation of salivary drug concentrations with plasma, especially when oral contamination was eliminated.

Several methods have been described for the collection of mixed saliva (whole). Many researchers have found it advantageous to further stimulate salivation and a number of stimuli have been used which include chewing paraffin wax, rubber bands and i.e of teflon (Dawes et al, 1999) once the samples have been collected, it is important that they be properly stored unless analysis are to be performed immediately (Chen et al, 1999).

The mechanism of drug transfer from blood to saliva include.

1. Passive trancellular diffusion
2. Ultrafiltation (or paracellular transport)
3. Active transport
4. Pinocytosis

Among the four known mechanism for drug transfer from blood to saliva via Salivary gland, there is no evidence that pinocytosis plays role, however the other three mechanisms are known to be involved (Lan and Mahmud, 1982).

In passive transcelluar diffusion highly lipid soluble material may cross capillary wall, with lipid layer of the epithelia cell wall providing the rate-limiting barrier. The Salivary concentration of the lipid soluble, un-conjugated steroids like oesriol and testosterone approximate the unbound plasma concentration. (Vining and Mccinley, 1999). Small polar molecules such as glycerol and sucrose enter into saliva.

Active transport mechanism clearly operate for many electrolytes and some proteins such as IgA. This mechanism also applies for some drugs like lithium (Idowu and Caddy, 1999).

The secretion of penicillin and tetracycline into Saliva appeared to be dependent upon the concentration in the blood (Borzelleca, 1999)

2.1.7 ASSAY METHOD

Several methods for the quantitative determination of drugs in pharmaceutical dosage forms and in biologic fluids are available

A rapid method for the determination of paracetamol in blood serum samples by first-derivative UV absorption spectroscopy was reported (Damiani et al, 1995). According to the method, serum (1ml) was deproteinised. Following centrifugation, 1ml of the

supernatant solution was treated with 0.2ml, 10M NaoH. The UV absorption spectrum was recorded and the first derivative at 292nm was calculated. Paracetamol was determined from a calibration graph of first-derivative intensity versus the original paracetamol concentrations in serum samples. The calibration graph was linear from 100-1500µg/ml of paracetamol.

Other anti-inflammatory drugs except salicylate did not interfere. Recoveries of the method ranged from 97.7-107%.

Garba et al, (1999) reported a UV method to determine the concentration of paracetamol in human saliva. According to the method a calibration graph of absorbance vs paracetamol concentration in saliva was plotted as follows: 5ml of ethyl acetate was added to each of 12 saliva samples containing different concentrations of paracetamol (i.e.0.00, 10.00, 20.00, 30.00, 40.00 and 50.00 µg/ml concentrations) in duplicate. The mixtures in the tubes were then vortex mixed for 1 minute at 2500rpm for 5 minutes. Ethyl acetate layer (upper) was removed from each of the tube using a Pasteur pipette and its absorbance measured at 262nm, using 1cm silica cuvette, with a double beam spectrophotometer. The absorbance for the blank (drug-free) saliva sample were then subtracted from those of the samples containing serial concentrations of paracetamol in order to obtain a set of absorbance reading corresponding to the serial concentration of paracetamol in saliva samples. The calibration curve was constructed by plotting the total absorbance readings obtained against the corresponding concentrations of paracetamol (10-50 µg/ml) in saliva samples. The graph was then used to determine the concentration of ingested paracetamol from saliva. Percentage recoveries ranged from 96-104%.

Assay of paracetamol and its metabolites in urine, plasma and saliva of children with chronic liver disease was reported (AL-Obaidy et al, 1995). Urine, plasma or saliva containing 10µg/ml 3-acetaminophen. (internal standard) were anlysed by HPLC on a 5µm sphensorb ODS-2 column (25cm x 4.6mm 1D) and UV detection. The mobile phases were acetonitrile/10mM-H2PO4 (1:24) of PH 3.5 for urine, methanol/acetonitrile/10mM H2Po4 (1:2:47) of PH 3.2 for plasma and a solvent similar to that described by Sommers et al (Human Toxicol (1987) 6,407) but containing 6% acetonitrile and not methanol for saliva. Calibration graphs were linear for 0.625-40µg/ml paracetamol and four of its metabolites in urine.

2.1.8 PARACETAMOL INTERACTIONS.

ANTICHOLINERGICS.

Anticholinergics can delay the onset of action of paracetamol. Anticholinergics slow gastric emptying thus reducing the rate of paracetamol absorption from the intestine.

Clinical Significance: The rate of absorption of paracetamol is considerably slower in the presence of propantheline, but the extent of paracetamol absorption is not affected. Other agents with anticholinergic activity such as tricyclic antidepressants antihistarnines and phenothiazines also probably delay acetaminophen absorption.

Tramadol:

Tramadol at a dose of 1mg/kg have no effect on paracetamol absorption (Murphy et al, 1997). However at a dose of 1.25mg, it has measurable but smaller effect by delaying absorption and may have clinical and economic advantages in acute pain management compared with conventional painkillers. (Crighton et al, 1998).

Barbiturates: Barbiturates are known enzyme inducers and they appear to enhance the metabolism of paracetamol. Results showed a lower oral paracetamol bioavailability and shorter serum half-life following IV administration of anti convulsants in epileptic patients (barbiturates, primidone, carbamazepine, phenytoin) (Perucca and Richen, 1979).

Clinical significant: There is an expected reduced therapeutic response to paracetamol based on these interactions. Phenobarbitone increases the hepatotoxicity and nephrotoxicity of paracetamol overdose in rats (Hansten and Horn, 1989), although evidence from humans is left to isolated case reports (Boyer and Rouff, 1971).

Caffeine: Enhances the analgesic effect of paracetamol.

Diazepam: Paracetamol may reduce the bioavailability of diazepam.

Clinical significant: The interaction is of no clinical significance as such no special precaution is necessary during co administration of diazepam an acetaminophen (Philip and John, 1989).

Food: Food, especially rich in carbohydrates, has been shown to delay the paracetamol absorption, an effect associated to delayed entry of the drug into the intestine or to delay in tablet disintegration or dissolution (McGilveray and Mattok, 1972)

Clinical significance: For rapid onset of analgesia paracetamol should be taken on an empty stomach.

Activated charcoal in large oral doses (5-10g) (Levy and Houston, 1976) and oral cholestyramine (Hansten and Horn 1989) were reported to reduce the GIT absorption

of paracetamol. Activated charcoal is therefore used to reduce absorption in poisoning.

Metoclopramide: Fasten the onset of the effect of paracetamol. Metoclopramide fasten the absorption of paracetamol by stimulating gastric emptying. The rate but not the extent of absorption is affected. (Nimmo et al, 1973)

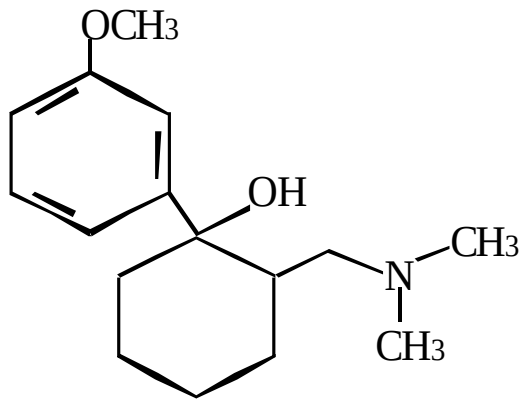
Clinical significance: No special precaution necessary during co-therapy with metoclopramide and acetaminophen (Philip and John, 1989).

2.2 TRAMADOL

INTRODUCTION AND CHEMISTRY

Tramadol Hydrochloride is a centrally acting analgesic. Chemical name for tramadol hydrochloride is ( ) cis- 2-(dimethylamino)methyl}-1-(3-methoxyphenyl cyclohexanol hydrochloride.

STRUCTURE



Tramadol

The molecular weight of tramadol hydrochloride is 299.8. It is a white, bitter, crystalline and odorless powder. It is readily soluble in water and ethanol and has a pka of 9.41. The water / n- octanol partition coefficient is 1.35 at PH7.

2.2.1 PHARMACOKINETICS

ABSORPTION.

Tramadol is rapidly and almost completely absorbed after oral administration. The mean absolute bioavailability of a 100mg oral dose is about 75%. Tramadol can be administered without regard to meals because food does not affect the rate or extent of its absorption. The mean peak(±SD) plasma concentration of racemic tramadol occurs at approximately 2 hours after a single oral dose in healthy subjects. (Dayer et al, 1997).

The separate {+} and {-} enantiomers of tramadol generally follow a parallel time course in plasma after a single 100mg oral dose. Following a 100mg oral administration of tramadol the maximum plasma concentration of the {-}= enantiomer are somewhat lower than those of the {+}- enantiomer (148±33 vs 168± 36ng/ml respectively). The{-}-M1 enantiomer is present at slightly higher plasma concentrations than the {+}-M1 enantiomer.

Plasma concentrations of racemic tramadol are predictable over a 50mg to 100mg single dose range. This is also true under multiple dose conditions. Steady state is achieved after two days of dosing tramadol by a 100mg 4 times daily regimen.

DISTRIBUTION

Tramadol is characterised by low plasma protein binding (20%) and quite extensive tissue distribution (apparent volume of distribution about 31l/kg). (Klotz and Fischer, 2003).

In another study the volume of distribution was 2.6 and 2.9l/kg in male and female subjects respectively following a 100mg intravenous dose. The binding of tramadol to

human plasma proteins is approximately 20% and binding also appears to be independent of concentrations up to 100mcg/ml. Saturation of plasma proteins binding occurs only at concentrations outside the clinically relevant range. Tramadol has been shown in rats to cross the blood- brain barrier.

METABOLISM.

Elimination of tramadol is primarily by hepatic route (metabolism by CYP2B6 to an active matabolite and CYP3A4 and CYP2B6) and partly by the renal route (up to 30% of dose). Elimination half-lives of the active agents range between 4.5 and 9.5h and total plasma clearance is moderately high (600ml/min). (Klotz and Fischer, 2003). Tramadol is extensively metabolized after oral administration. Approximately 30% of the dose is excreted in the urine as unchanged drug, whereas 60% of the dose is excreted as metabolites. The remainder is excreted as either as unidentified or an unextractable metabolites. The major metabolic pathways are N- and 0-demethylation and glucuronidation. (Ogunleye, 2001).

There is also sulfation in the liver. Only one of the metabolites (mono-O-desmethyltramadol denoted M1) is pharmacological active. Product of M1 is also dependent on the CYP2D6 iso enzyme of cytochrome P- 450.

After administration of 14C-tramadol to four rats and dogs, unchanged tramadol and a total of 24 metabolites, consisting of 16 phase 1 metabolite and eight conjugates(seven glucuronides, one sulfate) were isolated and tentatively identified, which accounted for more than 52% of the dose in urine of both species. Of the metabolites, five (M1-5) were previously identified. The metabolites were formed via six metabolic pathways. O-demethylation, N-demethylation, cyclohexyloxidation, oxidative N- dealkylation, dehydration and conjugation. The invitro metabolism of

tramadol was conducted using rat hepatic S9 fraction in the presence of an NADPH-generating system. Unchanged tramadol (30% of sample) plus nine metabolites, M1-7, tramadol-N-oxide (M31) and OH- cyclohexyl-M1 (M32), were profiled and tentatively identified based on MS and MS/MS data. (Wu, 2001).

EXCRETION

Approximately 30% of drug is excreted as unchanged in the urine, whereas 60% is excreted as metabolites. Elimination is therefore partly by the renal route (Klotz and Fischer, 2003). Elimination half- lives of the active agent range between 4.5h to 9.5h and total plasma clearance of tramadol is moderately high (600ml/min). The elimination kinetics can be described as 2- compartmental, with a half life of 5.1 hours for tramadol and 9 hours for the M1 derivative after a single oral dose of 100mg. This explains the approximately 2- fold accumulation of the parent drug and its M1 derivative that is observed during multiple dose treatment with tramadol (Dayer et al, 1997).

The mean terminal plasma elimination half lives of racemic tramadol and racemic M1 are 6.3±1.4 and 7.4±1.4 hours respectively. (±)- Tramadol effectively inhibits micturiton in conscious rats by stimulating μ- opioid receptors. A synergy between opioid receptor stimulation and monoamine reuptate inhibition may contribute to the micturiton effect. (Raj Kumar, 2003). Impaired renal function results in a decreased rate and extent of excretion for tramadol and its active metabolite M1. In patients with creatinine clearance less than 30ml /min there is need for dose adjustment.

2.2.2 PHARMACODYNAMICS

Tramadol is a centrally acting synthetic analgesic compound. Although its mode of action is not completely understood from animal tests at least two complementary mechanisms appear applicable. The two mechanisms are binding to μ-opioid receptors and inhibition of reuptate of norepinephrine and serotonin.

The selectivity of tramdol to μ-receptors has recently been demonstrated and the M1-metabolite of tramadol produced by the liver O-demethylation shows a higher affinity for opioid receptors than the parent drug. Nevertheless, this affinity of M1 for μ receptors of CNS remains low, being 6000 times lower than that of morphine. In contrast to other opioid, the analgesic action of tramadol is only partially inhibited by the opioid antagonist naloxone, which suggests the existence of another mechanism of action. This was demonstrated by the discovery of a monoaminergic activity that inhibits noradrenaline and serotinin (5-hydroxytryptamie 5HT)reuptate, making a significant contribution to the analgesic action by blocking nociceptive impulses at the spinal level. (±)-Tramadol is a racemic mixture of 2enantiomers each one displaying differing affinities for various receptors. (±)-Tramadol is a selective agonist of μ-receptors and preferentially inhibits serotonin reuptate, where as (-)-tramadol mainly inhibits noradrenaline reuptake. The action of these 2 enantiomers is both complementary and synergistic and results in the analgesic effect of (±)-tramadol (Dayer, 1997). The action of tramadol on the monoaminergic reuptake is similar to that of antidepressant drugs. It was found that (±)tramadol and (-)-tramadol have antidepressant-like effect in mice, probably mediated by the noradrenegic system rather than the serotoninergic or opioidergic ones. (Rogers – corrales et al, 1998). Result of sorne studies suggest that activation of opioid μ-receptors by tramadol can

increase the utilization of glucose and /or decrease hepatic gluconeogenesis to lower plasma glucose in diabetic rats lacking insulin, (Cheng, et al 2002). The lack of tolerance development of (3H)-5- HT uptake, together with the absence of behavioral alterations after chronic tramadol treatment, suggest that tramadol has an advantage over classical opioids in the treatment of pain disoders (Giusti et al, 2002). Tramadol also inhibit catecholamine secretion partly by inhibiting nicotinic ACHR-function in a nalogone insensitive manner and α-7 receptors are one of those inhibited by tramadol (Munehiro et al, 2002). Due to the monoamine reuptake inhibition an increase in transmission may result, triggering off excitatory phenomena with spike and wave activity in the CNS. Such excitatory effects, however, may only be seen when tramadol is used in doses exceeding the therapeutic range (Freye et al, 1998). At doses within the analgesic range,(±). Tramadol and its enantiomers induced anti convulsant effect in kindled rats. (Heidrun et al, 2000)

2.2.3 INDICATIONS

Tramadol is mainly indicated for the management of moderately severe pain. There is a positive trend that tramadol might improve the quality of intravenous regional anaesthesia (Tan et al, 2001).

2.2.4 TOXICITY AND ADVERSE EFFECTS

Estimates of ingested dose in foreign fatalities have been in the range of 3 to 5g. A 3g intentional overdose in patient in the clinical studies produced emesis and no sequelae. The lowest dose reported to be associated with a fatality was possibly between 500 and 1000mg in a 40kg woman. Serious potential consequences of

overdosage are respiratory depression and seizure. Other effects are dizziness, nausea, constipation headache, vomiting, anxiety and agitation. (Nossol et al, 1998).

Although tramadol can produce drug dependence of the μ-opioid type (like codeine) and potentially may be abused, there has been little evidence of abuse in foreign clinical experience. However in a study tramadol was shown to induce a stastically significanct increase in dopamine release within the NAc shell, which was maintained for at least 120min post treatment. This provides evidence that tramadol may posses greater abuse potential than originally believed (Sprague Jon et al, 2002). Naloxone is used to control some of the adverse events.

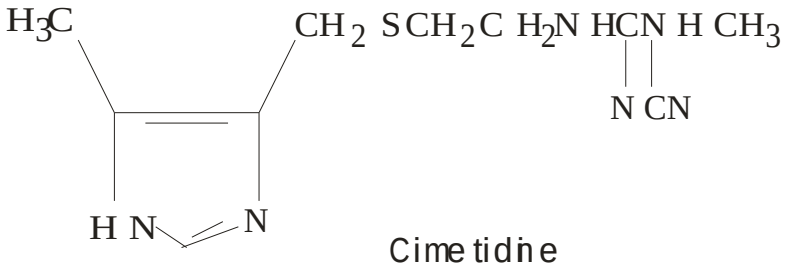
2.2.5 DRUG INTERACTION.

Tramadol is a mild inducer of selected drug metabolism pathways measured in animals. Carbamazepine causes a significant increase in tramadol metabolism. Quinidine causes an increase in concentrations of tramadol by enzyme inhibition. Concomitant administration of tramadol with cimetitine does not result in clinically significant changes in tramadol pharmacokinetics.

2.3 CIMETIDINE

2.3.1 INTRODUCTION AND CHEMISTRY

Cimetidine is 2-cyano-l-methy 1-3-(2-(5-methy) imidazol-4-ethyl)-guanidine; N-cyano-N-methyl-N"- (2)((5-methyl-1-imidazol 4-yl) methyl) thio)-guanidine.



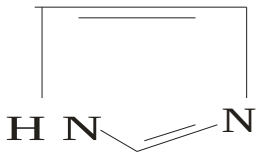
Cimetidine

Cimetidine is a white or almost white crystalline powder with an unpleasant odour. It has a melting point in the range 139 to 144°C.

The pka = 6.8

It is soluble in 1 in 200 of water, 1 in 18 of ethanol,1 in 1000 of chloroform, insoluble in ether. It is practically insoluble in dichloromethane and in ether. It dissolves in dilute mineral acids. The solubility of cimetidine in water is increased by the addition of dilute hydrochloric acid. At 37oC cimetidine is soluble 1 in 88 of water (The pharmaceutical codex, 1994).

Chemically cimetidine is a substituted imidazole compound.



Imidazole ring

Cimetidine is a weak base with a high degree of water solubility as shown above. The increased solubility in dilute acid is because it protonates the imidazole ring.

TABLE 2.3.1.1. Lists some of the physicochemical properties of cimetidine.

PHYSIOCHEMICAL CHARACTERISTIC OF CIMETIDINE		
1.	Appearance:	White to off-white crystalline powder.
2.	Polymorphism:	Polymorphic and exist in three polymorphic forms.
3.	Odour:	Odourless or with a faint odour
4.	Mol. Formular:	$C_{10}H_{16}N_6S$
5.	Mol. Weight:	242.34
6.	Melting point:	$141 – 143^0C$
7.	Pka:	6.80
8.	pH – Value:	pH of a 5.0mg/ml in carbondioxide free water is 8.0-9.5 (basic)
9.	Loss on drying:	When dried to constant weight at 100^0C to 150^0C, loses not more than 0.5% of its weight.
10.	Solubility:	Solubility in acetonitrile is 0.27% at 24^0. Slightly soluble in water (1.14% at 37^0C) , but Hydrochloride is more water soluble. Very soluble in methanol (14.1% at 37^0C). Practically insoluble in dichloromethane and in ether. Cimetidine dissolve in dilute mineral acids.
11.	Stability:	Stable for 48 hours at room temperature when added to commonly used i.v. solutions (e.g. 5% Dextrose injection)

Cimetidine hydrochloride has been found to be both visually and chemically stable for at least 1 week at ambient room temperature when combined with commonly used IV fluids (Rosenberg et al, 1980; Yuhas et al, 1981). Cimetidine hydrochloride when added to 5% dextrose and then frozen for 30 days was found to be stable and sterile for at least 8 days after thawing when kept at 4° (Walker et al, 1981).

2.3.2 History and development

Cimetidine was the prototypical histamine H_2-receptor antagonist from which the later members of the class were developed. Cimetidine was the culmination of a project at Smith, Kline & French (SK&F; now GlaxoSmithKline) to develop a histamine receptor antagonist to suppress stomach acid secretion.

At the time (1964) it was known that histamine was able to stimulate the secretion of stomach acid, but also that traditional antihistamines had no effect on acid production. In the process, the SK&F scientists also proved the existence of histamine H_2-receptors.

The SK&F team used a rational drug-design structure starting from the structure of histamine - the only design lead, since nothing was known of the then hypothetical H_2-receptor. Hundreds of modified compounds were synthesised in an effort to develop a model of the receptor. The first breakthrough was N^{α}-guanylhistamine, a partial H_2-receptor antagonist. From this lead the receptor model was further refined and eventually led to the development of burimamide - the first H_2-receptor antagonist. Burimamide, a specific competitive antagonist at the H_2-receptor 100-times more potent than N^{α}-guanylhistamine, proved the existence of the H_2-receptor.

Burimamide was still insufficiently potent for oral administration and further modification of the structure, based on modifying the pKa of the compound, lead to the development of metiamide. Metiamide was an effective agent, however it was associated with unacceptable nephrotoxicity and agranulocytosis. It was proposed that the toxicity arose from the thiourea group, and similar guanidine-analogues were investigated until the ultimate discovery of cimetidine.

2.3.3 PHARMACOKINETICS

Absorption

Cimetidine is readily abosorbed from the GIT after oral administration, it has a bioavailability of between 60-70%. Cimetidine plasma profiles from oral administration under fasted conditions often show a secondary maximum that is not observed when the drug is administered with a meal (Bodemar et al, 1979).

The two plasma level maxima typically occur between 1 and 2 hours and between 3 and 4 hours after oral cimetidine administration. Given the relative short elimination half –life and the fact that this double peak time frame coincides with intestinal transit time, variable absorption rate down the length of the intestine is anticipated to contribute to drug plasma level observations. Enterohepatic circulation (Veng Pederson and Miller, 1980), intestinal bacterial reconversion of biliary metabolite (Gugler et al, 1981), variable gastric emptying (Oberle and Amidon, 1987) and regional dependent absorption (Hui et al 1994) have all been proposed to account for these observations (Piyapolrungroj et al, 2000). But more importantly a decreased rate of absorption in the jejunum as compared with lower small intestine may contribute to plasma level double peaks and variable absorption (Piyapolrungroj et al, 2000). The first plasma level peak is generated by cimetidine absorption as the drug

enters the proximal small intestine from the stomach. Then after a slowing of the absorption rate in the jejunum coincident with systemic drug elimination, a secondary increased rate of drug absorption in the lower small intestine may generate the second plasma level peak.

Cimetidine is absorbed paracellularly and intracellular uptake is mediated by PH – dependent and potential – dependent processes in parallel with facilitative uptake across lateral membrane lining the paracellular pathway. Paracellular transport is regulated by intracellular cimetidine concentrations as a function of intracellular uptake and metabolism (Piyapolrungroj et al 2000).

In another study (Logan et al 1978) showed a good correlation between gastric emptying rate of a liquid meal and cimtidine absorption at 1 hour in healthy subjects. This suggests that the individual variation in gastric emptying was responsible for the variation in cimetidine absorption.

The bioavailability of cimetidine is significantly reduced by antacids, by up to one third, metoclopramide also reduced the biavailability by an average of 22% (Gugler et al 1981).

Distribution

Cimetidine is widely distributed and has a volume of distribution of about 1 litre /kg and is weakly bound, about 20%, to plasma proteins.

Cimetidine distributes widely and extensively throughout most body tissues. There is an extensive up take of cimtidine into selected organs (eg kidney, lungs) and tissues (eg lung). (Schentag et al, 1981). Cimetidine distributes into the cerebrospinal fluid (CSR) at a ratio of 0.1 to 0.2 compared to plasma. Higher ratios have been observed

in patients with liver disease (Schentag et al, 1981). This explains some of the adverse effects observed in patients with liver disease.

Cimetidine is concentrated in red blood cells (Somogyi et al 1980).The uptake into erythrocytes is much greater than that into both CSF and saliva. The mean saliva to plasma ratio is 0.2 (Somogyi and Gugler, 1983).

Between plasma concentration of 0.05 and 50 μg/ml, cimetidine was found to be between 18 and 26.3% bound to plasma proteins (Taylor et al, 1978). Cimetidine is transported across the human placenta bidirectionally at a rate about one third that of antipyrine and the transport is a passive one (Schenker et al, 1987).

In a study by Howe et al (1981), it was reported that the ratio of foetal to maternal blood concentrations of cimetidine was time dependent. The highest concentration of cimetidine in infant blood occurred with-in 1 hour after administeration.

Cimetidine is also secreted into breast milk and may reach the infant in several milligrams daily (Somogyi and Gugler, 1979).

Elimination

The elimination half-life from plasma is about 2hours and is increased in renal impairment (Gladziwa and Klotz, 1983).

Cimetidine is eliminated in the body by renal, metabolic and biliary routes. The principal route of elimination is however the renal route (Granen et al, 1979).

Cimetidine is secreted by the renal organic cation transport mechanism and that it probably undergoes passive reabsorption (non ionic diffusion) to a modest extent when the urine is alkaline. The relatively long half-life of the drug in the body, despite its very high renal clearance, is attributable to the large volume of distribution (Weiner and Roth, 1981). Between 50 and 80% of the intravenous dose is recovered in urine as unchanged cimetidine. The fraction is less after oral doses, but is

independent of the amount of dose. The high urinary excretion of cimetidine coupled with low plasma concentration results in a high renal clearance of the drug (Somogyi and Gugler, 1983). Biliary excretion fo cimetidine accounts for only 2% of the dose (Gugler et al, 1981). The biliary elimination is therefore negligible and of no clinical significance. In another study a mechanism for transport and microsomal metabolism of cimetidine in the jejunum is proposed (Piyapolrungroj et al 2000).

Cimetidine is mainly metabolised to cimetidine – S oxide in the Jejunum. Earlier report indicated that cimetidine s-oxide appearance in the jejunal lumen was greater in rats than humans (Hui et al, 1994). However, surface area to volume considerations indicate that cimetidine elimination in the human jejunum is substantial.

In the liver, cimetidine is metabolised to 3 known products, Cimetidine sulphoxide, hydroxymethyl cimetidine and guanyl cimetidine, although the latter may be an in-vitro degradation product (Taylor et al 1978). In man metabolism represents only 25-40% (depends on age) of the total elimination of cimetidine, the sulphoxide being the major metabolic (10-15%) and the hydroxy methyl derivative being the minor metabolite (4%) (Somogyi and Gugler, 1983). In a study (Henderson GI et al, 1988), it was found that the aged rat liver exhibits impaired cimetidine metabolism, resulting in decreased overall systemic clearance of the drug despite normal net renal tubular secretion, 2. There is no age – related enhanced sensitivity to cimetidine of the hepatic microsomal oxidising system using aminopyrine as the probe drug and (3) the large inhibition of aminopyrine metabolism in aged rats following various dose of cimetidine is due to decreased overall cimetidine clearance, resulting in higher concentrations of the inhibitor in the liver of aged rats (Schenker, et al, 1987).

Elimination of cimetidine is accelerated by an average of 15% in the presence of phenobarbitone due to induction of its metabolism by phenobarbitone (Somogyi et al,

1981). Elimination half life of cimetidine is approximately 2 hours in healthy adults with normal kidney and hepatic function (Somogyi and Gugler, 1983). Half-life increases in renal and hepatic impairment, and in the elderly.

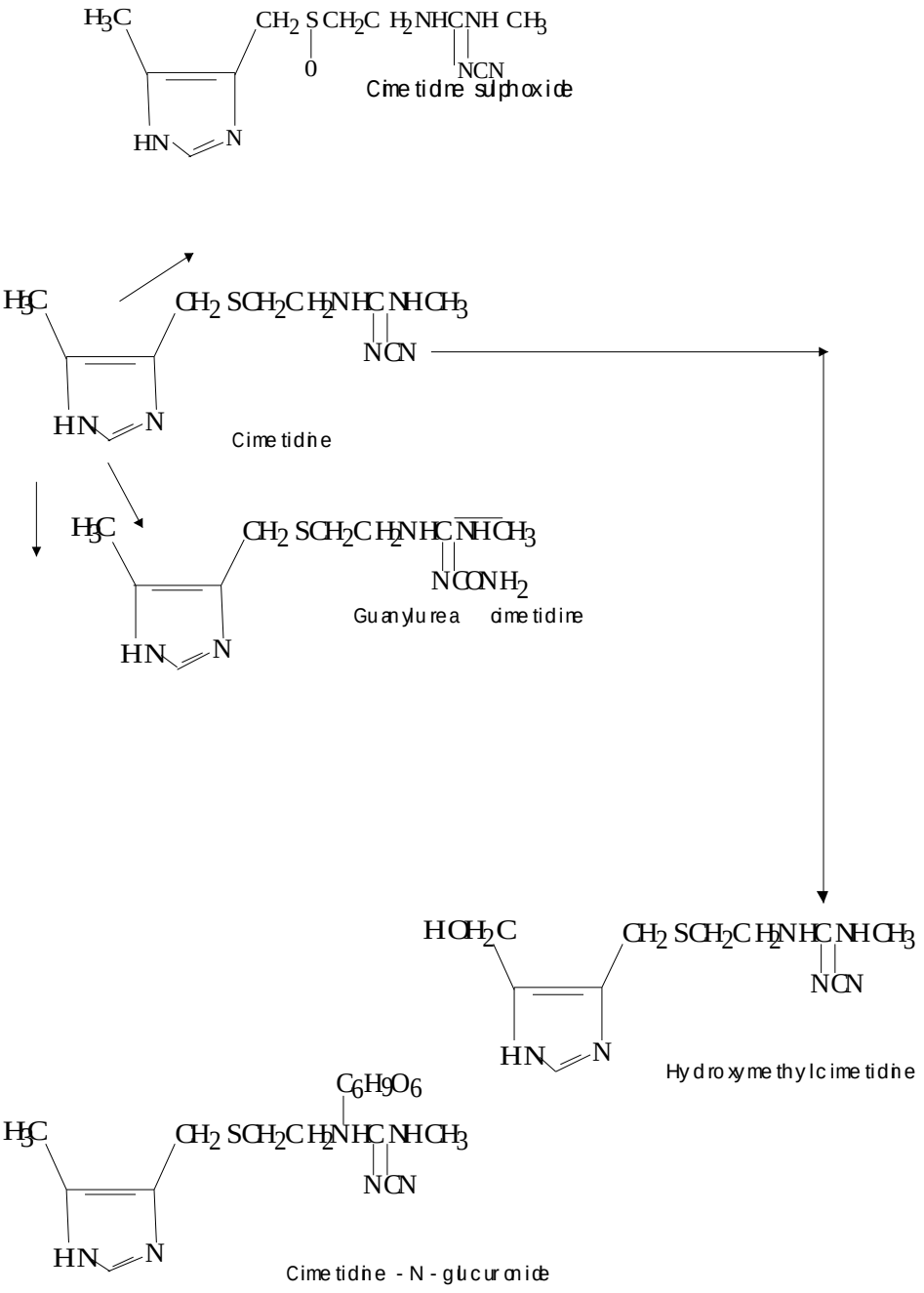


Figure 2.3.3.1: Schematic representation of the metabolic pathways for cimetidine.

2.3.4 PHARMACOLOGICAL PROPERTIES AND INDICATION

Cimetidine is a hitamine H2-receptor antagonist used in conditions where inhibition of gastric acid secretion may be beneficial, such as:- duodenal and gastric ulcers

- gastro – oesophageal reflux disease
- Selected cases of persistent dyspepsia
- Pathological hypersecretory states such as zollinger – ellison syndrome
- Stress ulceration
- Patients at risk of acid aspiration
- During general anaesthesia
- Cimetidine may also be used to reduce malabsorption and fluid loss in patients with the short bowel syndrome and to reduce the degradation of enzyme supplements given to patients with pancreatic insufficiency

2.3.5 ADMINSTRATION AND DOSAGE

In management of duodenal and gastric ulcers single daily dose of 800mg by mouth at bedtime is recommended, which should be given initially for at lease 4 weeks in the case of duodenal ulcers and for at lease 6 weeks in the case of gastric ulcers. A maintenance dose of 400mg may then be given once daily at bedtime. In gastro-oesophageal reflux disease the recommended dose is 400mg by mouth four times daily (with meals and at bedtime) for 4 to 8 weeks. In zollinger – Ellison syndrome, a dose of 300mg or 400mg by month four time daily is used, sometimes higher dose may be necessary.

The usual dose of cimetidine by intravenous injection is 200mg which should be given slowly over at least 2 minutes and may be repeated every 4 to 6 hour (Martindale, 1996).

2.3.6 ADVERSE EFFECTS.

Adverse reactions are generally infrequent and are usually reversible following a reduction of dosage or withdrawal of therapy. The commonest side-effects are diarrhoea, dizziness, tiredness, headache and rashes. Reversible confusional states, especially in the elderly or in seriously ill patients such as those with renal failure have occasionally occurred.

It has a week anti-androgenic effect and gynaecomastia and impotence have occasionally occurred in men receiving high doses.

Other adverse effects reported rarely are hypersensitivity reactions and fever, agranulocytosis or neutropenia and thrombocytopenia, interstitial nephritis, hepatotoxicity and cardiovascular disorders like arrhythmias.

2.3.7 DRUG INTERACTIONS

Cimetidine is a known inhibitor of many iso-enzymes of the cytochrome P-450 enzyme system (specifically CYP1A2, CYP2C9,CYP2C19, CYP2D6, CYP2E1 and CYP3A4). This inhibition forms the basis of the numerous interactions that occur between cimetidine and other drugs (Wikipedia 2006). Only few drugs have been reported to affect the action of cimetidine but many drugs can be affected by cimetidine.

Drugs affecting cimetidine

Single dose studies have shown reduced bioavailability of cimetidine due to antacids (Gugler et al, 1981). The results indicate that cimetidine and antacids should not be given together.

Metoclopramide may reduce the bioavailability of cimetidine possibly due to reduction of gastro-intestinal transit time (Gugler et al, 1981; Barzaghi et al, 1989). The prokinetic agent cisapride has been shown to have a similar effect on cimetidine (Kirch et al, 1989). Propantheline delays gastric emptying and reduces intestinal motility and has been reported to reduce the bioavailability of cimetidine (Kanto et al, 1981).

Drugs affected by cimetidine

The majority of these interactions are due to binding of cimetidine to cytochrome P-450 in the liver with subsequent inhibition of microsomal oxidative metabolism and increased plasma concentration of drugs metabolised by these enzymes.

Significant or potentially significant interaction have occurred with antiarrhythmic agents such as lignocaine and procainamide, sulphonyl urea and biguanide antidiabetic agents, antiepileptics such as phenytoin and carbamazepine, chloramphenicol, chlorpromazine, syclosporin, nifedipine, opioids such as pethidine, suxamethonium, theophylline, amitriptylline, urapidil, vasopressin and warfarin and other anticoagulants. The plasma concentrations of these drugs need to be monitored if they are used concomitantly with cimetidine (Martindale, 1996). (Garba et al, 1999) also report reduced plasma concentration of paracetamol when used with cimetidine due to reduced absorption, but increased AUC due to metabolic inhibition.

Cimetidine can inhibit tubular secretion of procainamide increasing the plasma concentration of the drug and its cardio-active, metabolite N-acetylprocainamide. Such interactions may require either a reduction of dosage or alteration of regimen. Cimetidine also interacts with some vitamins, mineral and food in a variety of ways.

Iron

Stomach acid may facilitate iron absorption H2-receptor blocker drugs reduce stomach acid and are associated with decreased dietary iron absorption (Aymard et al, 1988). People with ulcers may also loose blood through bleeding. Iron levels in the blood should therefore be checked regularly in ulcer patients.

Magnesium

In healthy volunteers, a magnesium hydroxide/aluminium hydroxide antacid taken with cimetidine decreased cimetidine absorption by 20 -25 % (Bachmann et al, 1994). People can avoid this interaction by taking cimetidine two hours before or after any aluminium/magnesium – containing antacids, including magnesium hydroxide found in some vitamin mineral supplements.

Vitamin B12

Hydrochloric acid is needed to release vitamin B12 from food so it can be absorbed by the body. Cimetidine, which reduced stomach acids, may decrease the amount of vitamin B12 available for the body to absorb (Salom et al, 1982).

Vitamin D

Cimetidine may reduce vitamin D activation by the liver.

Caffeine

Caffeine is found in coffee, tea, soft drinks, chocolate, guarana (paullinia cupana), and non prescription over – the-counter drug products, and supplement products containing caffeine or guarana. Cimetidine may decrease the clearance of caffeine from the body, causing increased caffeine blood levels and unwanted actions (Threlkeld, 1998).

Table: 2.3.7.1 Influence of cimetidine on the pharmacokinetic of drug (BNF, 2005)

DRUG CLASS	**INFLUENCE OF CIMETIDINE**	**REMARK**
Analgesics	Increase plasma concentration of opioid analgesics notably pethidine by inhibiting metabolism	N.P.H
Anti-arrythmics	Increase plasma concentration of amiodarone Flecinide, Lignocaine, procainamide, proafenone and quinidine.	P.H
Antibacterials	Redueces absorption of cefpodoxine. Increases Plasma concentration of metronidazole by inhibiting its metabolism	P.H
Anticoagulants	Enhances anticoagulant effect of incoumalone and warfarin by inhibiting their metabolism	P.H

Atidepressants	Increase plasma concentration of amitriptyline, desipramine, doxepin, imiprmine, nortriptyline, moclobemide and probably other antidepressants by inhibiting their metabolism	N.P.H
Antiepileptics	Increase plasma concentration of phenytion and carbamazepine by inhibiting their metabolism	N.P.H
Antifungals	Reduce absorption of itraconazole and ketoconazole. Increase plasma concentration of terbinafine.	N.P.H
Anxiolytics and Hypnotics	Increase plasma concentrations of benzodiazepines and chlormethiazole by inhibiting their metabolism	N.P.H
Antimalarials	Increase plasma concentrations of quinine and chloroquine by inhibiting their metabolism	N.P.H
Antipsychotics	Enhances effect of chlorpromazine clozapine and possibly other antipsychotics.	N.P.H
Beta-Blockers	Increase plasma concentrations of labetalo and propranolol by inhibiting their metabolism	N.P.H
Calcium-channel blockers	Increase plasma concentration of some calcium-channel blockers by inhibiting their metabolism.	P.H

CYCLOSPROIN	Possibly increase plasma cyclosporine concentration.	P.H
Cytotoxics	Increase plasma concentration of fluorouracil	N.P.H
Mebendazole	Enhance plasma concentration by inhibiting its metabolism	N.P.H
Antihistamines	Possibly increase concentration of loratadine	N.P.H
Ativirals	Plasma concentration of cimetidine possibly increased by amprenavir cimetidine possibly increase plasma concentration of zakitabine	N.P.H
Doperminergics	Increase plasma concentration of pramipexole	P.H
Sildenafil	Increase plasma concentrations.	P.H
Theophyline	Increase plasma concentrations.	P.H

KEY:

P.H = Potentially Hazardous Interaction.

N.P.H = Not Potentially Hazardous Interaction.

AIM AND OBJECTIVES OF THE STUDY

Previous studies have shown that cimetidine delays and reduces the absorption of paracetamol when the paracetamol is given 1 hour after cimetidine. The mechanism of this action was not well understood. This study will therefore attempt to find out whether tramadol will simulate the action of cimetidine thereby giving some insight into the mechanism of the cimetidine action.

It is well known that analgesics are combined for various reasons. Tramadol and paracetamol are combined sometimes in the same tablet due to their complimentary kinetics. This study will attempt to find out the pharmacokinetics of such a combination in our environment.

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1.1 CHEMICALS AND STANDARD SAMPLES

- Methanol, Analar, BDH Chemicals, England
- Ethyl acetate, May and Baker, England
- Distilled water
- Chewable parafilm
- Perchloric acid, Analar, BDH Chemicals, England
- Glacial acetic acid, Harris reagents, England
- Sodium hydroxide pellets, May and Baker, England
- Hydrochloric acid, Analar, BDH Chemicals, England
- Acetone, May and Baker, England
- Lead Nitrate, May and Baker, England
- Crystal Violet, Harris reagent, England
- Mercuric acetate solution (5%)

Standard pure sample of parcetamol used was obtained as gift.

Standard paracetamol powder;

Paracetamol Tablets

SOURCE: Purchased from a registered pharmacy.

BRAND NAME: Panadol

MANUFACTURER: Sterling Products (Nig.) PLC

51, Town Planning Way,

Ilupeju Industrial Estate, Lagos.

MANUFACTURE DATE: 01 2004

EXPIRY DATE: 01 2007

STRENGHT: 500mg per Tablet

BATCH NO: OO9E

NAFDAC REG NUMBER: 04-0205

TRAMADOL CAPSULES

SOURCE: Purchased from a registered pharmacy.

BRAND NAME: TRAMAL

MANUFACTURER: Grunenthal GMBH -52099 Aachen Germany.

MANUFACTURE DATE: 03 2003

EXPIRY DATE: 02 2007

STRENGHT: 50mg

BATCH NO: 197G11

NAFDAC REG NUMBER: 04-0515

CIMETIDINE TABLETS

SOURCE: Purchased from a registered pharmacy.

BRAND NAME: TAGAMET

MANUFACTURER: Smith Kline Beecham (Nig.) PLC

MANUFACTURE DATE: 01 2003

EXPIRY DATE: 12 2005

STRENGHT: 400mg

BATCH NO: 3001

3.1.2 GLASS WARES

- 10ml extraction tubes, pyrex, England
- Pipettes- 0.02ml, 0.1ml, 1ml, 5ml, 10ml, pyrex, England
- Measuring cylinder- 5ml, 10ml, 100ml, pyrex, England
- Screw capped sample bottles: 5ml, 10ml, 20ml
- Conical flasks: 25ml, 50ml, 250ml, Pyrex, England
- Test tubes: 10ml and 20ml, Pyrex, England
- Volumetric flasks, 25ml, 50ml, 250ml, Pyrex, England
- Beakers: 50ml, 100ml, 250ml, Pyrex, England
- Syringes and Needle : 5ml, 10ml, with 21G needle
- Crucible
- Round bottom flask, Pyrex, England
- Glass funnel and filter paper, Whatman
- Plastic stirring rod
- 20ml burette, pyrex, England
- Glass weighing pan

3.1.3 EQUIPMENT

- Auto-vortex mixer, stuart, England
- 800 Electric centrifuge, B. Bran Scientific and Instrument co. England
- Flask shaker, Gallenkamp, England
- Disintegration rate study apparatus, Erweka, England
- Hot air oven, Gallenkamp, England
- Electronic balance, Mettler, AE 240
- Thermocool refrigerator, Premeir, Nigeria

- UV Spectrophotometer, Jenway 6305, UV/Visible
- Melting point Apparatus Gallenkamp, England
- Dissolution rate study apparatus, Erweka, England
- Injection micro syringe, Hamilton, England
- Water bath, compenstat, Gallenkamp, England
- Weighing balance, Denver instrument XP 300.

3.2 METHODS:

3.2.1 QUALITY CONTROL

I. IDENTIFICATION TESTS

1. Identification Tests for Paracetamol Powder (BP2002)

a. Melting point – 170ºC

b. 1ml of hydrochloric acid was added to 0.1g of the powder and heated to boiling for 3mins, then 10ml of distilled water was added and cooled. No precipitate was formed. 0.5ml of 0.0167m potassium dichromate was added. A violet colour developed which did not change to red.

2. Identification for Paracetamol Tablets

A quantity of the powdered tablet containing 0.5g of paracetamol was extracted with 20ml of acetone. The resulting solution was filtered and evaporated to dryness. This was dried at 150ºC. The residue was then subjected to the tests.

c. Meeting point – About169ºC.

d. 0.1g of the residue was subjected to the same test as in B above and gave the same result.

3. Identification Tests for Cimetidine Tablets (WHO, 1991)

One tablet of cimetidine (containing 400mg) was weighed and the amount (0.081g) equivalent to 60mg cimetidine weighed and divided into 2 equal parts. The 2 parts were subjected to the following tests:

A. One part of the test substance was ignited. Lead nitrate paper was then exposed to the vapour evolved and observed for the darkening of the lead nitrate paper.

B. To the second portion was added 10ml of water and stirred. Few drops of potassium iodobismuthate/acetic acid test solution was added and observed for an orange precipitate.

II. TEST FOR UNIFORMITY OF WEIGHT

BP (2002) procedure was followed:

Twenty (20) tablets of Paracetamol were randomly selected and their average weight determined. The tablets were then weighed individually and percentage weight deviation of each tablets from mean (average) weight calculated.

The same procedure was repeated for cimetidine tablets. The results for the test are presented in chapter 4.

III. ASSAY FOR CONTENT OF ACTIVE INGREDIENT

1.Assay for Paracetamol Tablets

Twenty tablets of paracetamol were weighed and powdered. 0.177g of the powder containing 0.1g of paracetamol was added to 50ml of 0.1M sodium hyroxide, diluted with 100ml of water and shaken for 15mins, and sufficient water added to produce 200ml. This was then mixed, filtered and 10ml of the filtrate diluted to 100ml with water. 10ml of the resulting solution was added to 10ml of 0.1M sodium hydroxide and diluted to 100ml with water and the absorbance of the resulting solution measured at the maximum at 257mm.

2. Assay for Cimetidine Tablets

Non-aqueous titrimetric method, using 0.1M perchloric acid as titrant was adopted to assay for content of active ingredient of the cimetidine tablet. 20 tablets of cimetidine (400mg) were randomly selected and weighed. The tablets were then crushed to fine powder: powder (0.345g) equivalent 0.25g of cimetidine was weighed and dissolved by gentle warming and cooling in 30ml glacial acetic acid. The content was then titrated with 0.1M perchloric acid using 5% crystal violet dissolved in glacial acetic acid as indicator for end point determination.

The titration was performed 5 times and the average calculated.

3. Assay for tramadol capsules

Four capsules of tramadol were opened carefully into a clean crucible and 0.3g of powder which is equivalent to 0.15g of tramadol was weighed out and transferred into a dried conical flask. This was then dissolved in 40ml of glacial acetic acid and 10ml of mercuric acetate solution (5%). The resulting solution was well shaken and titrated with 0.1M Perchloric acid using crytal violet as indicator for end point determination.

The result of the assay is presented in chapter 4.

IV. DISINTEGRATION RATE TEST

The test was performed for paracetamol and cimetidine tablets

Six tablets were placed in 6 tubes (one tablet per tube) of the tablet disintegration apparatus (Erweka, Germany).

The assembly of tubes was suspended in a beaker containing the disintegration medium (0.1MHCl) that was maintained at 37ºC +-1ºC by an electrically heated water bath. The assembly of tubes was made to move up and down in the medium so that

the tablets were constantly agitated. The time taken for all the tablets to pass freely through the mesh at the lower end of the tubes is the disintegration time.

The results are presented in chapter 4.

V. Dissolution Tests

The rotary basket method described in the BP 2002 was adopted.

One tablet of paracetamol was placed in the basket and the basket placed in the round-bottom flask containing 1L of 0.1M hydrochloric acid maintained at temperature between 36.5 and 37.5ºC by an electric heater. The stirrer was set at 100 r.p.m. This was allowed to run for 45 minutes at which point 10ml of the dissolution medium was withdrawn, filtered and assayed using a UV-spectrophotometer at wavelength 257nm. The content of tablet released after 45 minutes was calculated by comparing the absorbance of the dissolution medium with the absorbance given by a known concentration of paracetamol in 0.1M hydrochloric acid at 257nm.

The percentage of paracetamol released after 45 minutes

$$= \frac{\text{Amount of sample released}}{\text{Content of standard paracetamol tablet}} \times 100$$

3.2.2 ANALYTICAL METHOD

The analytical method of (Garba et al, 1999) was adopted and modified. The method uses ehtylacetate as solvent, with UV-spectrophotometry at λmax of 262nm.

3.2.3 PRECISION OF THE ANALYTIC METHOD

The precision of the analytic method was determined by assaying a number of aliquots homogenous samples (50µg/ml and 20µg/ml) paracetamol in saliva in order to calculate statistical valid estimate of means among individual tests applied. This is repeated five times with estimates of standard deviation S.D and coefficient of variation C.V, both with in day and between day precision for building confidence in the application of the analytic method.

3.2.4 EXTRACTION PROCEDURE AND PERCENTAGE EXTRACTION RECOVERY

The method used for the extraction was adopted from (Garba et al, 1999). A 2ml of saliva sample was spiked with 25ul of the stock solution to get a working concentration of 50µl/ml. To this was added 5ml ethylacetate in glass Stoppard centrifuge. The mixture was then vortex mixed for 1minute and centrifuged at 2500rpm for 5 minutes. The upper layer was then removed using a Pasteur pipette and the absorbance taken at 262nm. The procedure was repeated with 0.1NHcl to determine percentage extraction recoveries. The procedure was repeated using 20ug/ml concentration.

% Extraction Amount recovery =

$$\frac{\text{Amount recovered after sample extraction}}{\text{Amount recovered after extraction with 0.1NHCl}} \times \frac{100}{1}$$

3.2.5 PREPARATION AND VALIDATION OF CALIBRATION CURVE

Preparation of Paracetamol Stock Solution

A stock solution of pure paracetamol powder in methanol with a concentration of 4mg/ml was used for the construction and validation of calibration curve. 100mg of pure paracetamol powder was accurately weighed on an electronic analytical balance. The powder was then transferred into a 25ml volumetric flask and dissolved with about 10ml of methanol by slow shaking. The volume was then made to 25ml with more methanol. The stock solution was then labelled and refrigerated pending its use.

Construction of Calibration Curve

A calibration curve of paracetamol in human saliva based on absorbance was prepared by spiking blank saliva with different volumes of standard solution of paracetamol to give a concentration range of 0 to 50µg/ml. Sufficient quantity of saliva was collected with the aid of chewing a piece of parafilm. The collected saliva samples were then pooled and then distributed into twelve 10ml extraction tubes (2ml per tube) using an auto pipette. Ten of the tubes were spiked with different volume of the stock solution in duplicate using a microHamilton syringe, to give different concentrations of the paracetamol. The remaining two were left as blank. Each concentration was spiked in duplicate (Table) and each duplicate was repeated four times on different days, resulting in 8 replica data for each concentration and blank. The volume of paracetamol stock solution spiked into the 2ml of saliva was determined by the formula.

$C_1V_1 = C_2V_2$

C_1 = Concentration of paracetamol stock

V_1 = Volume of stock solution to be spiked

C2 = Concentration ranges desired (0 -50µg/ml)

V2 = Volume of saliva samples (2ml)

Table 3.2.5.1 Shows the volume of stock solution of paracetamol spiked into 2ml of saliva and the concentration in saliva obtained.

To obtain absorbance readings for the various paracetamol concentration, 5ml of ethyl acetate were added to each of the twelve saliva samples in the centrifuge tubes. The tubes were then stopped with glass screw caps and each was shaken vigorously for one minute with a rotamixer. The tubes were then centrifuged for five minutes at 2500 revolutions per minute and the ethyl acetate layer removed with Pasteur pipettes. The absorbance reading of the ethyl acetate layer was obtained for each sample at 262mm wave length from a UV spectrophotometer. The absorbances of the blank samples were subtracted from the absorbance of those containing different concentration of paracetamol. The result is shown in **Table 4.4.1.1** And was used to construct the calibration curve by plotting the total mean absorbance (**Figure 4.4.1.1**) readings obtained against the concentration of paracetamol. The correlation co-efficient was determined with the aid of a computer programme.

Table 3.2.5.1 Volumes of stock solution spiked to saliva samples and the concentrations obtained for the construction of the calibration curve.

Serial No	Volume of stock solution spike to 2ml saliva sample (µl)	Concentration of paracetamol in saliva sample obtained (µg/ml)
1	0.00	0.00
2	0.00	0.00
3	5.00	10.00
4	5.00	10.00
5	10.00	20.00
6	10.00	20.00
7	15.00	30.00
8	15.00	30.00
9	20.00	40.00
10	20.00	40.00
11	25.00	50.00
12	25.00	50.00

Validation of the Calibration Curve

The constructed calibration curve was validated by spiking four (4) pairs of 2ml blank saliva with different volumes of paracetamol stock solution to give four (4) pairs of different concentration from the ones used for the calibration curve. Each sample was then extracted with 5ml ethyl acetate and the absorbance corresponding to the various spiked concentration obtained at 262nm, wavelength from a UV spectrophotometer. The absorbance data obtained were then converted directly into concentration from

the calibration curve. The concentrations obtained were then compared with the actual concentration spiked to the saliva samples.

The results are presented in chapter 4.

3.2.6 IN VIVO PHARMACOKINETIC STUDIES

Eight healthy male volunteers aged between 25 – 38kg (31.50 ± 4.34) years weighing between 60 – 75kg (66.25±5.62) participated in the study. The volunteers were free from liver and kidney diseases, they were non-smokers and non-drinkers and counseled to abstain from taking drug or kolanut for at least two weeks before the commencement of and during the study. They were also well counseled on the study in simple language and their informed consent obtained before the commencement of the study.

Crossover study was used. The protocol of the study was divided into 5 phases. The first phase of the study involved the ingestions of two tablets of panadol (1g of paracetamol) with about 150ml of distilled water after overnight fasting.

About 4ml saliva samples were immediately collected prior to the drug ingestion and at 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, and 6.00 hour intervals. The saliva samples were then refrigerated pending analysis.

The second phase involved the concomitant ingestion of 1g of paracetamol (2 panadol tablets) and 100mg of Tramadol (2 Tramadol 50mg capsule) with about 150ml of distilled after overnight fasting. Saliva samples were collected and stored as in the first phase of study.

In the third phase of the study 100mg of Tramadol was ingested with about 150ml distilled water, followed by 1g of paracetamol 1hr 45 minutes later. The saliva samples were taken immediately before the ingestion of the paracetamol and at 0.25, 0.500, 1.00, 2.00, 3.00, 4.00, 5.00 and 6hrs intervals as before .

The fourth phase of the study involved the concomitant ingestion of 1g of paracetamol and 400mg of cimetidine. Saliva samples were collected and stored as in the second phase,

The fifth phase of the study involved the ingestion of 400mg of cimetidine followed by 1g of paracetamol 1hour later. Saliva samples were collected and stored as in the third phase.

Extraction and Analysis

A 2ml of saliva samples were placed in 10ml extraction centrifuge tubes using an auto pipette. 5ml of ethyl acetate were then added to the saliva. The centrifuges were stopped with glass screw caps and were shaken vigorously for one minute with a Rota mixer. The contents were then centrifuge for five minutes at 2500 revolution per minute.

The ethyl acetate layer (upper layer) was then removed with a Pasteur pipette and their absorbance was measured at 262nm by a UV-spectrophotometer. The absorbance of the blank was subtracted from those of the samples containing paracetamol to give a set of absorbance reading from time zero to 6 hours.

Data Handling

The values for the concentration of paracetamol in saliva samples were plotted against time on a logarithmic scale. The method was then employed to determine the following pharmacokinetic parameters: Lag time, Absorption and elimination half-lives (t1/2ab andt1/2el respectively), Absorption and elimination constants (Kab and Kel respectively).

Other pharmacokinetic parameters were calculated as follows:

1. AUC (from time 0 to 6 hours) were calculated using the triangular-trapezoidal rule.
2. AUC (from time 0 to infinity) were calculated as follows:

   AUC (0 - ∞) = AUC (0 -6) + AUC (6 - ∞)

   AUC (6 - ∞) = $\frac{\text{Conc. At 6 hours}}{\text{Kel}}$

3.Volume of distribution (Vd) = $\frac{\text{F.D}}{\text{AUC.Ke}}$

4. Clearance (Cl) = Vd.Ke = $\frac{0.693.\ \text{Vd}}{\text{t1/2 el}}$

Where,

AUC = Area under the salivary concentration – time curve

Kel = Elimination rate constant

T1/2el =Elimination half life

F = Bioavailability (assumed to be 100%)

D = Dose of paracetamol ingested

All the mean pharmacokinetic parameters for the 8 volunteers in this study were analysed statistically using the student two tailed t-test for paired data with the aid of a computer program. Values of $P < 0.05$ were considered as significant $P > 0.05$ as not significant for the differences between the controls and treated groups.

CHAPTER FOUR

RESULTS

4.1 Quality Control Assessment

Table 4.1.1.1 Quality control assessment results for Paracetamol, Cimetidine and Tramadol tablet and capsules

S/NO	QUALITY ASSESSMENT	PARACETAMOL	CIMETIDINE	TRAMADOL	REMARK
1.	Identification	Positive	Positive	Positive	Pass
2.	Weight uniformity Mean (M.P.D)	0.5925 (2.1097)	0.5515 (3.2456)	-	Pass
3.	Assay for content Mean ± S.E.M	99.53±0.339	98.65±0.175	98.802	Pass
4.	Disintegration rate Mean	5.5	8.25	-	Pass
5.	Dissolution rate Mean ± S.E.M	85.46± 0.764	-	-	Pass

M.P.D. = Maximum percentage deviation

S.E.M = Standard error of the mean

4.2 VALIDATION OF ANALYTIC METHOD

Table4.2.1.1 Result for the validation of analytic method

SAMPLE	CONCENTRATION (µg/ml)	CV%	N
Within-day run Paracetamol	20 50	1.8 2.2	5 5
Between day run Paracetamol	20 50	1.5 2.6	5 5

4.3 PERCENTAGE EXTRACTION RECOVERY OF THE METHOD

Table 4.3.1.1 Result for the percentage extraction recovery

SAMPLE	CONCENTRATION (µg/ml)	RECOVERY %+ SD	N
PARACETAMOL	20	95±6.2	5
	50	95±5.5	5

4.4 CONSTRUCTION AND VALIDATION OF CALIBRATION CURVE

4.4.1 Calibration Curve

Table4.4.1.1 Calibration Data for spiked saliva samples containing paracetamol

CONCENTRATION (µg/ml)	MEAN ABSORBANCE	STANDARD DEVIATION	MEAN ABSORBANCE – BLANK
0	0.046	0.0321	0
10	0.285	0.0521	0.239
20	0.511	0.0324	0.465
30	0.726	0.0792	0.680
40	0.972	0.0612	0.926
50	1.256	0.0972	1.210

4.4.2 Validation Of Calibration Curve

Table 4.4.2.1 Result obtained from the validation of the calibration curve

Vol. of stock (mcl)	Absrob (nm)	Conc. Of pcm(mg/mi)	Conc. From Calibr. Curve (mcg/ml)	%recovery
0	0	0	0	0
2.5	0.12	5.0	4.9	98
7.5	0.361	15.0	15.2	101.33
12.5	0.545	25.0	26.2	104.60
17.5	0.842	35.0	36.0	102.57
22.5	1.021	45.0	43.6	96.67
72.5	1.262	55.0	52.6	97.45

4.5 IN-VIVO PHARMACOKINETIC STUDIES

Paracetamol concentrations under two protocols of concomitant and delayed administration of tramadol and cimetidine are presented in tables

Table 4.5.1.1

MEAN SALIVA CONCENTRATION OF PARACETAMOL ± SD FOR PARACETAMOL ALONE AND WITH TRAMADOL PHASE II & III (µg/ml)

Time (h)	PCM (Alone) PHASE I	With Tramadol concomitant PHASE II	With Tramadol delayed PHASE III
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.25	12.5 ± 1.96	12.54 ± 1.30	10.60 ± 2.14
0.5	18.34± 3.80	18.63 ± 3.46	16.05 ± 2.85
1.00	17.49 ± 2.74	17.25 ± 2.78	17.65 ± 2.44
2.00	13.61 ± 1.95	13.13 ± 2.11	14.14 ± 1.63
3.00	6.01 ± 1.29	5.84 ± 1.34	5.89 ± 1.38
4.00	3.19 ± 0.66	3.23 ± 1.26	2.70 ± 0.38
5.00	1.94 ± 0.38	2.0 ± 0.52	2.0 ± 0.39
6.00	1.16 ± 0.18	1.17 ± 0.14	1.06 ± 0.12

Table 4.5.1.2

G. MEAN SALIVA CONCENTRATION ± SD FOR PARACETAMOL ALONE AND WITH CIMETIDINE (CONCOMITANT) AND (DELAYED) PHASE IV & V

Time (h)	PCM (Alone) PHASE I	With Cimetidine Concomitant PHASE IV	With Cimetidine delayed PHASE V
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
0.25	12.5±1.96	12.57±1.19	3.9 ± 0.78
0.5	18.34± 3.80	18.08 ± 3.10	8.2 ± 1.86
1.00	17.49± 2.74	17.95 ± 3.22	12.5 ± 2.87
2.00	13.61 ± 1.95	13.28 ± 2.63	11.8 ± 3.46
3.00	6.01 ± 1.29	6.91 ± 1.19	10.5 ± 2.83
4.00	3.19 ± 0.66	3.16 ± 1.32	8.8 ± 2.97
5.00	1.94 ± 0.38	1.95 ± 0.83	5.8 ± 1.80
6.00	1.16 ± 0.18	1.15 ± 0.12	4.10 ± 1.33

4.6 PHARMACOKINETIC PARAMETERS

Mean ± S.E.M salivary pharmacokinetic parameters of paracetamol ingested alone, and for the concomitant and delayed administration protocols for tramadol and cimetidine.

Table 4.6.1.1

MEAN VALUES ± SEM OF PHARMACOKINETIC PARAMETERS OF PARACETAMOL GIVEN ALONE AND PARACETAMOL + TRAMADOL PHASE II

Pharmacokinetic Parameters	Paracetamol Alone (Phase I)	Paracetamol Concomitant With Tramadol Phase II	% Difference	P-value
C_{max} (µg/ml)	18.99 ± 1.247	19.1 ± 1.068	0.58%	P >.05
T_{max} (h)	0.75 ± 0.094	0.69 ± 0.091	8%	P >.05
$T_{1/2}$ ab (h)	0.306 ± 0.036	0.300 ± 0.030	1.96%	P >.05
K_{ab} (µg/ml /h)	2.55 ± 0.368	2.47 ± 0.035	3.14%	P >.05
$T_{1/2}$ el (h)	1.025 ± 0.074	1.030 ± 0.070	0.49%	P >.05
Kel (µg/ml /h)	0.698 ± 0.045	0.690 ± 0.048	1.15%	P >.05
Vd (l)	30.40 ± 2.984	31.13 ± 2.640	2.35%	P >.05
Clearance (l/h)	20.50 ± 1.018	20.93 ± 1.167	2.05%	P >.05
Lagtime(h)	0.131 ± 0.023	0.138 ± 0.016	5.07%	P >.05
AUC (µg.h/ml)	49.76 ± 2.421	48.91 ± 2.650	1.71%	P >.05

Table 4.6.1.2

MEAN VALUES ± SEM OF PHARMACOKINETIC PARAMETERS OF PARACETAMOL GIVEN ALONE AND GIVEN 1h 45mins AFTER TRAMADOL PHASE III

PHARMACOKINETIC PARAMETERS	PARACETAMOL ALONE (PHASE I)	PARACETAMOL WITH TRAMADOL PHASE III	% Difference	P-VALUE
C_{max} (µg/ml)	18.99 ± 1.247	17.83 ± 0.878	6.11%	P >.05
T_{max} (h)	0.75 ± 0.094	0.94 ± 0.063	20.21%	P <.05
$t_{1/2}$ ab (h)	0.306 ± 0.036	0.38 ± 0.021	19.47%	P <.05
K_{ab} (µg/ml /h)	2.55 ± 0.368	1.89 ± 0.095	25.88%	P <.05
$T_{1/2}$ el (h)	1.025 ± 0.074	0.89 ± 0.057	13.17%	P >.05
Kel (µg/ml /h)	0.698 ± 0.045	0.729 ± 0.047	4.25%	P >.05
Vd (l)	30.40 ± 2.984	30.02 ± 1.565	0.66%	P >.05
Clearance (l/h)	20.50 ± 1.018	21.28 ± 1.01	3.67%	P >.05
Lagtime(h)	0.131 ± 0.023	0.15 ± 0.016	12.67%	P >.05
AUC (µg.h/ml)	49.76 ± 2.421	47.59 ± 1.85	4.36%	P >.05

Table 4.6.1.3

MEAN VALUES ± SEM OF PHARMACOKINETIC PARAMETERS OF PARACETAMOL GIVEN ALONE AND PARACETAMOL + CIMETIDINE CONCOMITANT PHASE IV

PHARMACOKINETIC PARAMETERS	PARACETAMOL ALONE (PHASE I)	PARACETAMOL WITH CIMETIDINE PHASE IV	% Difference	P-VALUE
C_{max} (µg/ml)	18.99 ± 1.247	19.02 ± 1.122	0.16%	P >.05
T_{max} (h)	0.75 ± 0.094	0.81 ± 0.092	7.14%	P >.05
$t_{1/2}$ ab (h)	0.306 ± 0.036	0.325 ± 0.030	5.85%	P >.05
K_{ab} (µg/ml /h)	2.55 ± 0.368	2.30 ± 0.270	9.80%	P >.05
$T_{1/2}$ el (h)	1.025 ± 0.074	1.038 ± 0.038	1.25%	P >.05
Kel (µg/ml /h)	0.698 ± 0.045	0.674 ± 0.025	3.44%	P >.05
Vd (l)	30.40 ± 2.984	30.38 ± 2.286	0.06%	P >.05
Clearance (l/h)	20.50 ± 1.018	20.25 ± 1.084	1.22%	P >.05
Lagtime(h)	0.131 ± 0.023	0.138 ± 0.016	5.07%	P >.05
AUC (µg.h/ml)	49.76 ± 2.421	49.98 ± 2.700	0.44%	P >.05

Table 4.6.1.4

MEAN VALUES ± SEM OF PHARMACOKINETIC PARAMETERS OF PARACETAMOL GIVEN ALONE AND PARACETAMOL + CIMETIDINE DELAYED PHASE V

PHARMACOKINETIC PARAMETERS	PARACETAMOL ALONE (PHASE I)	PARACETAMOL WITH CIMETIDINE DELAYED PHASE V	% Difference	P-VALUE
C_{max} (µg/ml)	18.99 ± 1.247	13.03 ± 1.131	31.38%	P <.05
T_{max} (h)	0.75 ± 0.09	1.38 ± 0.183	45.65%	P <.05
$t_{1/2}$ ab (h)	0.306 ± 0.036	0.569 ± 0.044	46.22%	P <.05
K_{ab} (mg/ml/h)	2.55 ± 0.368	1.269 ± 1.240	50.24%	P <.05
$T_{1/2}$ el (h)	1.025 ± 0.074	2.519 ± 0.130	59.31%	P <.05
Kel (µg/ml/h)	0.698 ± 0.045	0.281 ± 0.015	59.74%	P <.05
Vd (l)	30.40 ± 2.984	57.69 ± 5.835	47.30%	P <.05
Clearance (l/h)	20.50 ± 1.018	16.01 ± 1.731	21.90%	P <.05
Lagtime(h)	0.131 ± 0.023	0.206 ± 0.015	36.41%	P <.05
AUC (µg.h/ml)	49.76 ± 2.421	70.53 ± 2.561	29.45%	P<.05

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 QUALITY CONTROL ASSESSMENTS.

IDENTIFICATION TEST:

The test carried for paracetamol standard powder showed conformity with BP2002 requirement. Paracemtol gave a violet colour with 0.0167M potassium dichromate solution. It also gave a melting point of 172ºC and BP 2002 specified 168-172^0 C. These tests verified the identity of the paracetamol powder.

The paracetamol tablets after extraction with acetone the residue gave the same violet colour with 0.0167M potassium dichromate solution and a melting point of 168^{0}C which is about 169^{0}C thereby conforming with BP2002 requirements, thus verifying the identity of the paracetamol tablets.

Cimetidine gave an orange precipitate with potassium iodobismuthate/acetic acid solution and when it was ignited under a lead nitrate Paper, the vapour darken the paper. These tests conform with the (WHO, 1991) requirement for cimetidine in the basic tests for pharmaceutical dosage forms.

UNIFORMITY OF WEIGHT

Test for the uniform of weight was carried out to ensure accurate administration of doses is achieved during the study. Mean weights for paracetamol and cimetidine tablets were found to be 0.5925g and 0.5515g respectively. The maximum percentage deviation of a paracetamol tablet from the mean weight was 2.1097 whereas the

maximum deviation for cimetidine tablets was 3.2456. This is in conformity with BP 2002, which states that for tablets of average weights of 250mg or more to pass the test, not more than 2 of the individual weight deviate from the mean weight by more than 5% and none deviate by more than twice that percentage. This shows that the samples have passed the test

Assay for content of active ingredient

Paracetamol tablets 500mg

The assay for paracetamol tablet gave 99.65% which is in conformity with BP 2002 which specified a range of 95%-105%. This test gave the assurance that the tablets used for the study contain the specified active ingredient.

CIMETIDINE TABLETS

The assay for cimetidine tablets gave 99.53% which is also in conformity with BP2002 and gave the assurance that the cimetidine tablets used contain the stated amount of active ingredient.

TRAMADOL CAPSULES

The assay for tramadol capsules gave 98.80%, which gave the assurance that the capsules used contain the required amount of active ingredient.

DISINTEGRATION TEST

The time taken for the cimetidine tablets to disintegrate was 8.25 minutes while that of paracemtamol was 5.5. This is in conformity with the BP2002 which gave a time of less than 15 minutes for uncoated Tablets. This test is important for this particular study because any delay in absorption will not be attributed to the quality of the

tablets, since they disintegrate in good time. Even though the in-vitro condition is not exactly the same in-vivo, it still indicate what happen in invivo

DISSOLUTION TEST.,

The percentage of paracetamol released from the paracetemol tablet after 45 minutes was 85.46%. This shows the tablet have passed the test. BP2002 states that not less than 70 % to be related after 45 minutes. Even though the experimental conditions are not the same with the in-vivo condition, the test indicates the tablet will dissolve in good time, thus assuring bioavailability.

5.2 VALIDATION OF ANALYTIC METHOD

Result for the validation of analytic was presented in **Table ……**

The analytic method has been validated based on the CV% obtained within day and between day run of the praracetamol. We were thus able to:

1. Build confidence in the analytic method
2. Sure of reproducibility of result

5.3 PERCENTAGE EXTRACTION RECOVERY

Result for the percentage extraction recovery was presented in **Table 4.3.1.1**

The extraction recovery percentage (Above 95%) gave assurance on the extraction method adopted. It is now assured that the active ingredient (paracetamol) is extracted in high percentage to facilitate efficient analysis.

5.4 CONSTRUCTION AND VALIDATION OF CALIBRATION CURVE

5.4.1 CALIBRATION CURVE

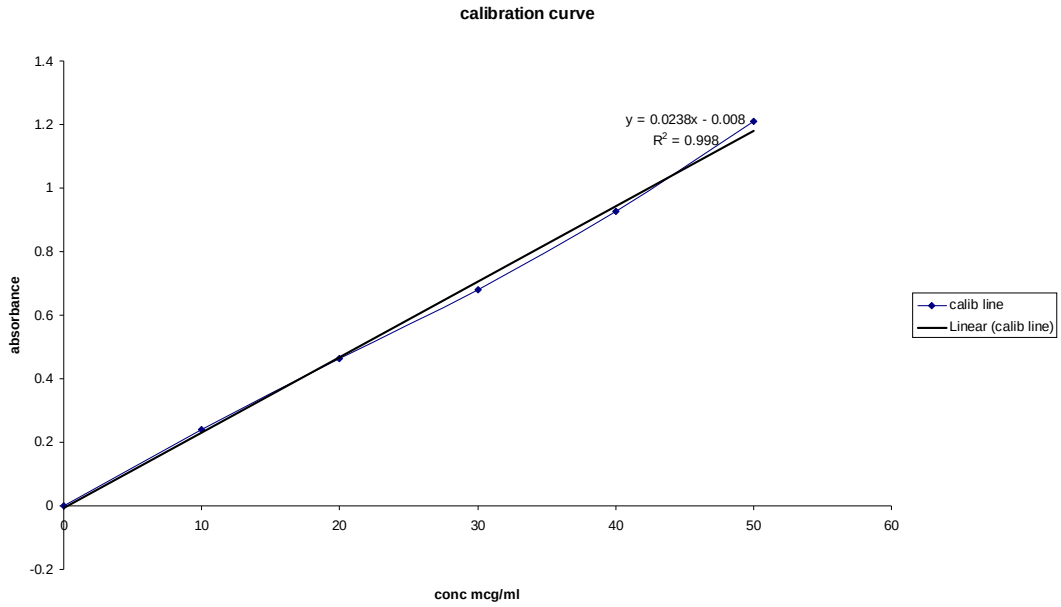
Linear calibration curve with good correlation coefficient (r = 0.998) of paracetamol in ethyl acetate over the range of concentrations 10-50µg/ml, using UV spectrophotometer is shown in F**igure 4.4.1.1**

The mean data obtained for construction of calibration curve are presented in T**able 4.4.1.1** the correlation coefficient of 0.998 shows a very good correlation of response of the detection system at the concentration of paracetamol in saliva used. This gave the assurance that an accurate estimation of the paracetamol in saliva will be obtained from the calibration curve.

Due to recovery losses and the variability inherent in measurement in biologic fluids, calibration curves using identical analytic parameter to be used for biologic sample are always required.

The curve is usually a plot showing the relationship between an independent variable X (usually drug concentration) and a dependent variable Y (some analytic measurement). For example concentration (x-axis(and absorbance (Y-axis

Fig: 4.4.1.1



5.4.2. VALIDATION OF THE CALIBRATION CURVE

Table **4.4.2.1** shows the data obtained for the validation of the calibration curve. The result obtained demonstrated a good correlation between the spiked concentrations and those estimated directly from the calibration curve.

The relative recoveries ranged from (96 – 104%) which is good enough for accurate quantitative determination of paracetamol in saliva samples in the concentration range of the calibration curve. The relative recovery is the measure of the accuracy of the assay method (Smith and Steward, 1981). In actual sense the relative recovery is a measure of how close the results obtained from the calibration curve are to the actual levels of drug in the spiked biologic samples.

5.5 IN – VIVO PHARMACOKINETIC STUDIES

Tables 4.5.1.1 and 4.5.1.2 Show the mean± SD salivary concentration – time data for paracetamol alone (control) and for the influence of Tramadol and cimetidine on

paracetamol under concomitant and delayed administration of the paracetamol. **Figures 4.5.1.1 and 4.5.1.2.** show the mean salivary concentration –time profiles for paracetamol alone (control) and for the influence of Tramadol and cimetidine on paracetamol under concomitant and delayed administration of paracetamol respectively.

5.5.1 SINGLE DOSE SALIVARY PHARMACOKINETICS OF PARACETAMOL

The mean pharmacokinetic data for paracetamol administered alone (phase I) is shown in **Table 4.5.2.1**

The pharmacokinetics of paracetamol has been extensively investigated (Thomas 1993, Bello 1990, Clements and Prescott 1982, Garba et al 1999). The results have revealed some inter individual variability. This is mainly attributed to individual variability in gastric emptying rate (Heading et al 1973). The t1/2ab of 0.306h is in close agreement with Bukhari (1997), it is however much higher than the 0.2656h reported by Garba et al (1999) all for concentrations in saliva. The time to peak concentration (Tmax) was 0.75h. This is lower than 0.88hr reported by Bukhari (1996). The value is however within the range of 0.5 – 1 hour reported by most established literature (Ameer and Greenblat 1997; Goodman and Gilman, 1996). The Cmax was 18.99µg/ml which is lower than 30.00µg/ml reported by Bukhari (1996) using saliva levels.

The lag time in this study was 0.131 hour, which is in close agreement with 0.1406 reported by Garba et al (1999). The elimination half life was found to be 1.025h, with corresponding elimination rate content (kel) of 0.698 µg/ml/hr. The t1/2 el is within

the range of 1-2h reported in most established literature (Goodman and Gilman, 1996; Payan and Katzung, 1995). Volume of distribution (Vd) was 30.40l with the corresponding clearance of 20.50L/h. These figures are in close agreement with Garba et al (1999), with a Vd of 29.1931L and a clearance of 18.1861L/h. The Vd is however lower than those reported by Bello (1990) and Bukhari (1996) with 40.75L and 43.012L respectively. The clearance is however much higher than the 12.07 and 12.904L/h reported by Bukhari(1996) and Bello (1990), respectively. The area under the curve from time 0 to infinity (AUC) was 49.76 µg.h/ml. this is much lower that the 128.98, 77.497 and 57.736 µg h/ml reported by Bukhari (1996), Bello (1990) and Garba et al (1999) respectively.

The results from this as well as previous studies showed a lot of inter individual variability in the pharmacokinetics of paracetamol between the different groups. Apart from interindividual differences in gastric emptying (Heading et al 1973), other factors such as age, bodyweight, environmental factors, disease state etc can contribute to the variations in the pharmacokinetics of paracetamol. In the study however the values are with in the ranges reported by most established literature.

5.5.2 INFLUENCE OF TRAMADOL ON THE PHARMACOKINETICS OF PARACETAMOL

The mean estimated salivary concentration – time data and the salivary concentration time profiles for paracetamol alone (control) and for the influence of Tramadol on

paracetamol under concomitant and delayed administration of paracetamol were presented. **Table 4.5.1.1** and **Figure 5.5.2.1**.

The meant $\pm$ S.E.M salivary pharmacokinetic data for paracetamol administered concomitantly with Tramadol is shown in table **4.5.2.1.**

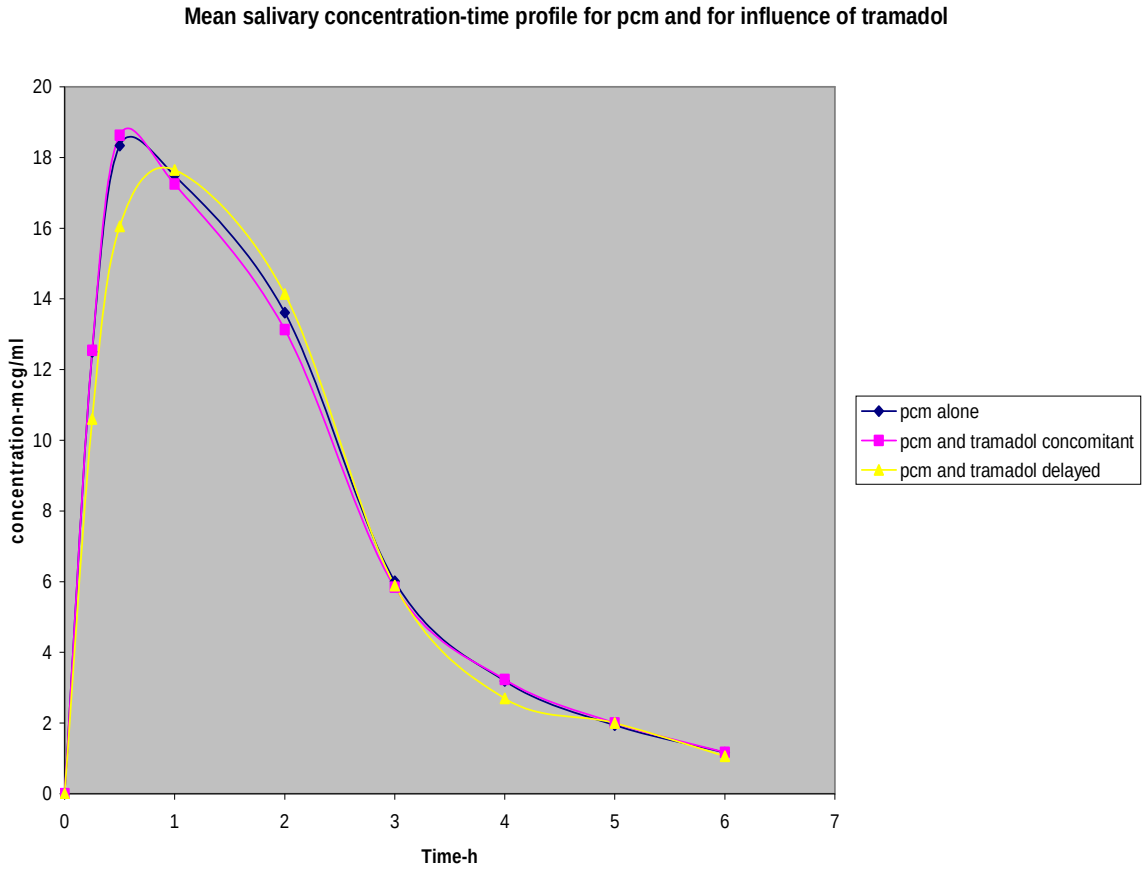


Fig: 5.5.2.1 Mean salivary concentration – time profiles for paracetamol alone and for the influence of tramadol under concomitant and delayed administration.

PCM = Paracetamol

When paracetamol was administered concomitantly with tramadol there were no significant changes (in the mean pharmacokinetic data(to paracetamol administered alone (P>0.05). These findings indicated that tramadol has no effect on the pharmakonetics of paracetamol when the drug is taken concomitantly with paracetamol. This result is expected based on the pharmacokinetics of the two drugs, because for a pharmacokinetic drug interaction to be observed a reasonable concentration of the two drugs must appear in plasma at the same time or tramadol must appear in plasma before the paracetamol to be able to influence the kinetics of paracetamol. The time to reach peak plasma concentration for paracetamol is between 10 – 60 minutes (Martindale, 1996) where as Tramadol is about 1.8 hs. Therefore, when paracetamol is administered concomitantly with tramadol, paracetamol will appear at reasonable concentration in plasma before the tramadol and its effects on pharmacokinetics of paracetamol will not be manifested.

When paracetamol administration was delayed by 1h 45mins after tramadol, there were significant changes in some of the mean pharmacokinetic data **(Table 4.5.2.3)**

Absorption parameters like Tmax and t1/2ab were significantly increased by 20.21% and 19.47% respectively, where as Kab was significantly reduced by 25.88%. Tramadol is known to stimulate the μ-opiod receptors like morphine, thereby causing a delay in gastric emptying, a phenomenon which reduces the absorption of paracetamol (Heading et al 1973). Although less potent than morphine on μ-opoid receptors, the effect seems to be dose dependent.

At 1mg/kg using electrical bioimpedence technique and the paracetamol absorption test, there is no effect (Murphy et al, 1997). At 1.2mg/kg using paracetamol absorption test, there is a measurable but insignificant effect (Crighton et al, 1998) while at 1.6mg/kg, there is a significant effect (present study). The elimination parameters like kel and clearance were increased by 4.25% and 3.67% respectively, where as t1/2el was reduced by 13.17%. These increases are expected because tramadol is a mild enzymes inducer. The increase are however statistically not significant ($p>0.05$) because the tramadol is a very mild enzymes inducer. The volume of distribution is only reduced by 0.66%, whereas the AUC is reduced by 4.36%. The reduced AUC indicated a slight increase in elimination.

These observations show that concomitant administration of paracetamol and tramadol have no effect on the pharmacokinetics of paracetamol. It can infact be an advantage combining the two drugs in one tablet, that is taking advantage of the quick action of paracetamol (quick onset of action) and long duration of action of tramadol (long lasting pain relief). The delayed administration of paracetamol 1h 45mins after tramadol shows a slight delay in absorption at high doses. Since the AUC and clearance are not significantly affected, the net outcome of treatment will not be clinically significant. Moreover, these changes are only observed at higher doses and at 95% confidence level.

5.5.3 INFLUENCE OF CIMETIDINE ON THE SALIVARY PHARMACOKINETICS OF PARACETAMOL

The mean estimated salivary concentration – time data and the salivary concentration time profiles for paracetamol alone (control) and for the influence of cimetidine on paracetamol under concomitant and delayed administration of paracetamol were presented in **Table 4.5.1.2** and **Figure 5.5.3.1**.

The meant $\pm$ S.E.M salivary pharmacokinetic data for paracetamol administered concomitantly with cimetidine is shown in table **4.5.2.1.**

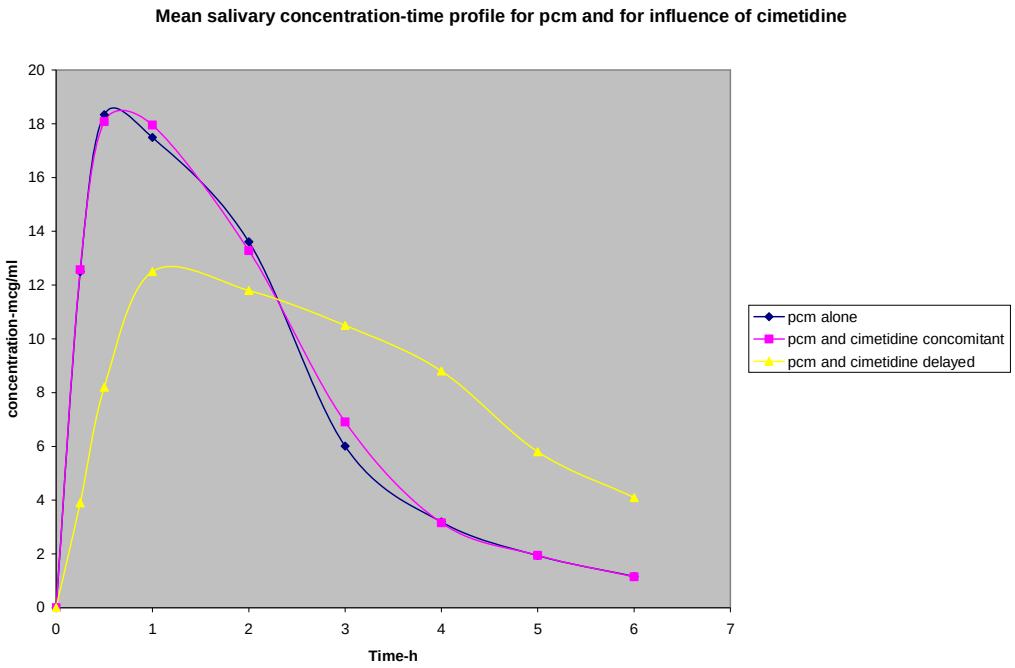


Fig 5.5.3.1 Mean salivary concentration–time profiles for paracetamol alone and for the influence of cimetidine under concomitant and delayed administration.

PCM = Paracetamol

When paracetamol was administered concomitantly with cimetidine there were no significant changes (in the mean pharmacokinetic data(to paracetamol administered alone (P>0.05). These findings indicated that cimetidine has no effect on the pharmakonetics of paracetamol when the drug is taken concomitantly with paracetamol. This result is expected based on the pharmacokinetics of the two drugs, because for a pharmacokinetic drug interaction to be observed, a reasonable concentration of the two drugs must appear in plasma at the same time or cimetidine must appear in plasma before the paracetamol to be able to influence the kinetics of paracetamol. The time to reach peak plasma concentration for paracetamol is between 10 – 60 minutes (Martindale, 1996) where as for cimetidine is between 1-2hs. Therefore, when paracetamol is administered concomitantly with cimetidine, paracetamol will appear at reasonable concentration in plasma before the cimetidine and its effects on pharmacokinetics of paracetamol will not be manifested.

When paracetamol administration was delayed by 1h after cimetidine, there were significant changes in the mean pharmacokinetic data **(Table 4.5.2.4)**

As shown in the table Cmax and Kab were significantly reduced by 31.38%and 50.24% respectively (p<0.05). Tmax, t1/2ab and Lag time were significantly increased by 45.65%, 46.22% and 36.41% respectively (p<0.05).

This study showed that cimetidine impaired the absorption of paracetamol under delayed administration of paracetamol 1h after the cimetidine.

The reduced absorption of paracetamol is most likely due to delay in gastric emptying (Heading et al, 1973). Nimmo et al (1973) also showed that propantheline reduced the rate of absorption of paracetamol by delaying gastric emptying. The effect of tramadol in the reduction of the absorption of paracetamol also supports the delay in

gastric emptying as the reason for cimetidine action on paracetamol. This is due to the action of tramadol on μ-opioid receptors which is known to delay gastric emptying. Some workers (Kwanashie et al, 1992) reported that cimetidine at higher concentrations (500 to 1000μg/ml) cause relaxation of the guinea pig ileum in vitro, a factor that can further explain the delayed absorption of paracetamol.

The clinical implication of the reduced rate of absorption of paracetamol by cimetidine, is that the absorption may be so slow that the minimum effective concentration (MEC) may never reached. The onset of action may also be delayed by the reduced rate of absorption, thus causing delayed onset of analgesia.

The t1/2el was significantly ($p<0.05$) increased by 59.34%, while kel was significantly reduced by 59.74%. The total body clearance on the other hand was reduced by 21.90% ($p<0.05$) and the corresponding volume of distribution (vd) was significantly increased by 47.30% ($p<0.05$). AUC was significantly increased by 29.45%. The reduced elimination, reduced clearance and increase AUC indicated that cimetidine inhibited the metabolism of paracetamol. But because inhibition of metabolism and impairment of absorption were taking place simultaneously, no increase in salivary concentration of paracetamol was observed. Cimetidine is a substituted imidazole, which are powerful inhibitors of metabolic drug oxidation through their interaction with microsomal cytodrome P-450 enzymes (Puurunen and Pelkonen, 1979). The increase in AUC (29.45%, $P<0.05$) despite reduction in absorption indicated that reduced clearance (metabolic inhibition) is very strong. Ziemniack et al (1986) also reported that cimetidine inhibited the metabolism of paracetamol. The increased AUC can also be as a result of more prolonged contact

with absorptive surface areas of the intestine due smooth muscle relaxation. This implied delayed absorption but increased extent of absorption.

The present study agree with Garba et al (1999), which revealed that paracetamol absorption was delayed by cimetidine, an effect that was attributed to the indirect action of cimetidine on gastric emptying and by its direct action on GIT smooth muscle.

Overall the study agrees with the pharmacokinetic profile of the three drugs and earlier works carried out on these drugs. However, the slight increase in the elimination of paracetamol after delayed tramadol need to be further studied especially with multiple dosing protocols to ascertain the real action after multiple dosing regimens.

5.6 CONCLUSION

Salivary concentration following ingestion of paracetamol is enough for pharmacokinetic studies. Due to the high degree of correlation with plasma concentration, the salivary concentration can be used to monitor drug levels in hospitals.

The method of extraction and analysis are relevant in this kind of study. These are based on the percentage recovery obtained and the overall result of the study.

From this study, it can be concluded that tramadol and cimetidine have no effects on the pharmacokinetics of paracetamol, when the either drug is administered concomitantly with paracetamol. However tramadol when administered 1h 45mins before paracetamol impaired the absorption of paracetamol to some extent. An action attributed to the action of tramadol on μ-opioid receptors, which causes delayed

gastric emptying. Under this protocol (delayed administration), tramadol also caused an insignificant increase in elimination of paracetamol. Tramadol is a mild enzyme inducer.

When paracetamol administration was delayed by 1h after cimetidine, the absorption and elimination of paracetamol are both impaired. This delay in absorption of paracetamol by cimetidine, we can now conclude to be due to delay in gastric emptying, while the delayed elimination is due to metabolic inhibition.

We can therefore recommend concomitant administration of paracetamol and cimetidine whenever the need arises.

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APPENDIX 1
ANALYSIS OF TABLES USED
UNIFORMITY OF WEIGHT
PARACETAMOL TABLET 500MG

S/NO	WEIGHT OF TABLE (G)	% DEVIATION FROM MEAN WEIGHT
1	0.590	0.4220
2	0.600	1.2500
3	0.580	2.1097
4	0.600	1.2500
5.	0.600	1.2500
6.	0.600	1.2500
7	0.590	0.4220
8	0.590	0.4220
9	0.600	1.2500
10	0.600	1.2500
11	0.580	2.1097
12	0.580	2.1097
13	0.600	1.2500
14	0.600	1.2500
15	0.580	2.1097
16	0.590	0.4220
17	0.600	1.2500
18	0.590	0.4220
19	0.580	2.1097
20	0.600	1.2500

MEAN WEIGHT =0.5925G

APPENDIX II
Cimetine tables 400mg

S/NO	WEIGHT TABLET (G)	% DEVIATION FROM MAN WEIGHT
1	0.550	0.2720
2	0.550	0.2720
3	0.570	3.2456
4.	0.540	2.0852
5	0.560	1.5179
6	0.550	0.2720
7	0.540	2.0852
8	0.556	1.5179
9	0.550	0.2720
10	0.550	0.2720
11	0.570	3.2456
12	0.540	2.0852
13	0.540	2.0852
14	0.550	0.2720
15	0.550	0.2720
16	0.550	0.2720
17	0.560	1.5179
18	0.550	0.2720
19	0.540	2.0852
20	0.560	1.5179

Mean Weight = 0.5515g

APPENDIX IIIA

ASSAY OF CONTENTS CIMETIDINE 400mg

S/NO	PERCENTAGE CONTENT	CONTENT (MG) PER TABLE OF FOUND	REMARK
1	98.90	395.6	PASS
2	98.40	393.6	PASS
3	98.50	394.00	PASS
4.	99.20	396.8	PASS
5	98.25	393	PASS
MEAN ±SEM	98.65 ± 0.175	394.6 ± 0.6 99	

APPENDIX III B

PARACETAMOL 500MG TABLE

S/NO	PERCENTAGE CONTENT	CONTENT (MG) PER TABLE OF FOUND	REMARK
1	99.18	495.90	PASS
2	100.58	502.90	PASS
3	98.63	493.15	PASS
4.	99.28	498.15	PASS
5	99.98	499.90	PASS
MEAN ±SEM	99.53 ± 0.339	498.0 ± 1.667	PASS

APPENDIX IV
DISSOLUTION TEST FOR PARACETAMOL TABLET 500MG

S/NO	PERCENTAGE CONTENT	CONTENT (MG) PER TABLET FOUND	REMARK
1	84.12	420.60	PASS
2	86.86	434.30	PASS
3	87.45	437.25	PASS
4.	85.40	427.00	PASS
5	83.47	417.35	PASS
MEAN ±SEM	85.46 ± 0.764	427.30 ± 3. 821	

APPENDIX V
PARTICULAR OF SUBJECTS USED FOR THE STUDY.

S/NO	CODE NAME	AGE (YEARS)	WEIGHT (KG)	SEX	REMARK
1	1A	30	65	MALE	HEALTY NON
2	AM	25	62	-	SMOKER
3	SA	25	64	-	-
4	AU	32	70	-	-
5	AM	36	72	-	-
6	AG	38	75	-	-
7	AA	32	62	-	-
8	DR	34	60	-	-

MEAN AGE 31.50± 4.34
MEAN WEIGHT = 66.25± 5.62

APPENDIX VI
DISTEGRATION RATE TEST

	PARACETAMOL		CIMETIDINE	
	DISINTEGRATION	REMARK	DISINTEGRATION	REMARK
S/N	TIME (MIN)		TIME (MIN)	
1	5.5	PASS	9.25	PASS
2	4.0	PASS	9.50	PASS
3	7.0	PASS	7.00	-
4	6.0	PASS	9.00	-
5	5.0	PASS	6.5	-
	5.5min		8.25min	

APPENDIX VII
DATA OBATINED FOR THE CONSTRUCTION FOR THE CALIBRATION CURVE

EXPERIEMENT	EXP2		EXP3		EXP 4			
CONC . OF STOCK SOLN OF PARACETAMOL IN SALIVA (µg/ml)	1A	1B	2A	2B	3A	3B	4A	4B	
0.00	0.050	0.042	0.040	0.058	0.056	0.033	0.037	0.052	
10.00	0.270	0.265	0.2925	0.280	0.313	0.252	0.290	0.318	
20.00	0.552	0.542	0.495	0.483	0.473	0.531	0.552	0.490	
30.00	0.752	0.740	0.694	0.769	0.715	0.750	0.685	0.703	
40.00	0.960	0.927	0.975	0.986	1.022	0.956	0.960	0.990	
50.00	1.232	1.209	1.262	1.194	1.307	1.278	1.250	1.316	

CONCENTRATION OF PARACETAMOL (SALIVA) FOR 8 VOLUNTEER WITH MEAN.

Time H	Mean (µg/ml)	A	B	C	D	E	F	G	H
0.25	12.5	12.5	11.8	9.0	15.5	12.8	12.8	12.5	14.5
0.5	18.34	16.7	13.0	14.2	16.6	23.6	19.5	21.0	22.4
1	17.49	16.2	15.2	13.4	18.0	16.7	19.6	22.2	18.6
2	13.61	14.3	12.2	10.0	15.2	13.5	14	16.5	13.2
3	6.01	4.5	15.8	4	7.2	7.8	6.2	6.8	5.8
4	3.19	2.2	2.4	3.2	3.5	2.8	3.8	4.0	3.6
5	1.94	1.8	1.9	2.0	2	1.2	2.4	2.4	1.8
6	1.16	1.1	1	1.2	1	1	1.5	1.3	1.2

CONCENTRATION OF PARACETAMOL (SALIVA) AFTER CONCOMITANT ADMINISTRATION WITH TRAMADOL (µg/ml)

Time H	Mean (µg/ml)	A	B	C	D	E	F	G	H
0.25	12.54	12.2	11.8	10.2	12	14.6	13.0	13.0	13.4
0.5	18.63	16.0	13.5	15.2	17.6	22.5	20.4	21.7	21.8
1	17.25	16.4	15.8	12.5	18.5	18.0	16.5	22.2	18.5
2	13.13	13.4	12	9	15.2	13.2	12.8	16.0	13.4
3	5.84	5.5	5.6	5.2	8	4.2	6.2	7.5	4.5
4	3.23	2.6	2.9	2.7	3	2.8	4.2	4	3.6
5	2.0	1.6	1.7	1.8	22	1.6	2.8	2	2.9
6	1.17	1	1	1.2	1.1	1.4	1.2	1.3	1.2

CONCENTRATION OF PARACETAMOL (SALIVA) AFTER DELAYED (1 H 45 MIN) ADMIN OF TRAMADOL.

Time H	Mean (µg/ml)	A	B	C	D	E	F	G	H
0.25	10.60	11.5	9.5	8.4	8.2	14.0	8.5	11.2	13.5
0.5	16.05	16.0	12.4	9.5	15.4	18.4	18.5	20.6	17.6
1	17.65	16.8	14.6	15.5	16.0	17.0	19.4	21.5	20.4
2	14.14	14	13.8	13.6	13.5	14.5	14	15.2	14.5
3	5.84	4.2	4.5	5.0	6.0	8	6.4	5.5	6.5
4	2.70	2	1.9	2.8	3.2	3.4	2.8	3	2.5
5	2.0	1.6	1.5	2.2	2.0	1.5	2.3	2.4	1.5
6	1.06	1.0	1.0	1.2	1.0	1	1.2	1.1	1

CONCENTRATION OF PARACETAMOL IN SALIVA AFTER CONCOMITANT ADMINISTRATION OF CIMETIDINE (µg/lm)

Time H	Mean (µg/ml)	A	B	C	D	E	F	G	H
0.25	12.57	12.5	12.2	10.5	11.5	14.2	13.4	13.5	12.8
0.5	18.08	15.8	13.8	15	17.5	18.5	20.2	21.8	22.0
1	17.95	16.5	15.5	13.2	18.0	22.4	17.2	22.6	18.2
2	13.28	13.6	11	8.5	15.4	14	12.5	17.0	14.2
3	6.91	5.2	5.5	5.6	7.5	5.2	6.5	7.6	4.2
4	3.16	2.2	3.2	2.4	3.2	2.5	3.8	4.5	3.5
5	1.95	1.4	1.8	1.5	2	1.6	2.6	2.5	2.2
6	1.15	1.0	1	1.2	1.1	1	1.3	1.2	1.0

CONCENTRATION OF PARACETAMOL IN SALIVA AFTER DELAYED ADMINISTRATION OF CIMETIDINE. (1H)

Time H	Mean (µg/ml)	A	B	C	D	E	F	G	H
0.25	3.9	3.8	3.4	2.8	3.5	3.6	4.0	5.2	4.8
0.5	8.2	6.2	5.8	6.5	8.5	8.6	9.4	10.2	10.6
1	12.5	9.5	8.6	10.4	11.2	16.5	14.8	14.5	14
2	11.8	8.4	7.5	11.5	9.4	15.2	12.2	15.6	16.5
3	10.5	7.2	6.6	9.2	8.4	13.5	11.2	12.5	13.8
4	8.8	5.8	5.4	6.2	7.0	12.6	10.2	11.2	11,8
5	5.8	3.5	3.6	4.8	5.0	8.2	6.4	7.2	7.5
6	4.10	2.4	2.5	3.4	3.8	6.2	4.5	5.2	4.8