CHAPTER II

REVIEW OF THE LITERATURES

History of Chemotherapy

The use by ancient Chinese of preparations obtained from plants and animals to treat wounds dated far back into the history of medicine. Undoubtedly these "folk medicines" often contained either chemicals which were themselves effective antibacterials or some fungi capable of producing agents responsible for the beneficial effects. The Chinese of those days certainly did not know the rationale behind the therapeutic effectiveness of these medicines, and their uses were entirely empirical. It was Paul Ehrlich who first began to put the whole process of antibacterial attack on a molecular basis so that it became more easily understood and more able to profit from its associations with organic chemistry and other biological sciences, knowledge of which were increasing rapidly at that time.

Ehrlich was fascinated by the selective actions of several stains and of dyes which are still used for histological examination of tissues. He reasoned that if certain chemicals could be drawn by the affinity to particular types of tissues, then it might be possible if those chemicals had antibacterial activity, to design agents which would be drawn to specific sites and there exert chemical actions to kill microbes. The outcome from a great deal of Ehrlich's works

has been the introduction of compounds designed to fix themselves very strongly to proteins, carbohydrates and fats, but which, in general, had a low selective capacity. For they bound and interfered with the activities of both the host cells and the cells of the invader. He found arsenic to be an effective agent for the treatment of syphilis but it was extremely toxic to man as well as to micro-organisms. In attempting to overcome this serious drawback, Ehrlich developed several complex arsenic derivatives with the hope that these chemicals may have high selective toxicity. Unfortunately his attempt was not successful, for these agents were still extreme--ly toxic to patients and it was questionable whether the treatment itself was not worse than the disease. At any rate we can say that Paul Ehrlich was the person who put forward the principles of antimicrobial chemotherapy on the scientific ground.(14)

After Ehrlich's death, a compound called Prontosil was discovered by chemists in Bohemia. It was a potent antibacterial agent and a powerful red tissue dye. Further investigations of this compound showed its antibacterial activity to reside on part of the molecule not associated with dying property. This finding in fact marked the beginning of the sulfonamides era. Once it was established that sulfonamides were the effective antibacterial agents, the concept of antibacterial agent being a dye had been ignored. All modified compounds synthesized later on were small organic molecules with absolutely no dyestaff properties and exert their antibacterial effect in a totally different manner.(14)

After the search for new antimicrobials through modification of organic compounds had lost its popularity, interest in this area had shifted to the possibility that one type of micro-organism may produce agents which hindered the growth of another. In the early 1920s. Fleming worked with a chemical known as lysozyme showed that this agent is capable of destroying certain types of bateria and therefore presumably has a potential therapeutic value. However, the greatest discovery in antimicrobial chemotherapy at the end of the 20s, when Fleming was working with the products of fungi which finally led to the isolation of the new antibacterial compound known as penicillin. This drug,produced by penicillium mold inhibits the growth of many micro-organisms, notably those of gram-positive species.(14)

The fact that an organism can produce agent which is a potent killer of other organism, and yet is non-toxic to human, has enormous impact on the pharmaceutical industry. Soon streptomycin, the tetracyclines and chloramphenicol were discovered. These new antibiotics can inhibit not only the growth of gram-positive but also some species of gram-negative micro-organisms. Thus, they were called "broad spectrum" antibiotics whereas penicillin was called narrow spectrum antibiotic.

CAP was first isolated in 1947 from a soil bacterium Streptomyces venezuelae. Subsequently, the antibiotic was prepared synthetically and marketed in 1949. CAP was the first "broad spectrum" antibiotic with activity against most gram-positive and many gram-negative bacteria, anaerobic bacteria, and rickettsiae. To date, this broad activity still remains.(1)

Physical properties and chemical structure

1. Physical properties

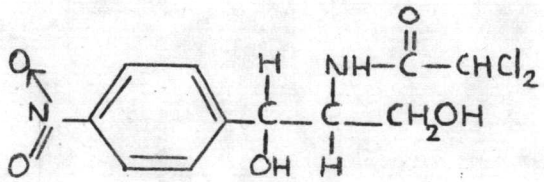
1.1 CAP is fine, white to greyish-white or yellowish-white odourless crystals (needles or elongated plates) with a very bitter taste. Its melting point is 149°C to 153°C.[16]

1.2 CAP is very lipid soluble and only sparingly soluble in water. Therefore, aqueous solubility must be achieved by attaching a polar group, usually an ester linkage, to the CAP molecule. Several esters are provided for intravenous and intramuscular uses, for instance, succinate, glycinate and phthalate ester.[1,37] However palmitate ester is for liquid oral preparation.[1]

1.3 Aqueous solution loses about half their CAP content by hydrolysis on storage for 290 days at 20°c to 22°c, under the same conditions, buffered solution (using borax) at pH 7.4 loses about 14%. The loss on heating the solutions at 100°c for 15 minutes is about 3%.[16]

1.4 CAP is destroyed by light in 0.35% aqueous solution and the solution becomes yellow and acid. The major degradation products are formed by oxidation, reduction, and subsequent condensation.[16]

2. Chemical structure



= Structure of Chloramphenicol

CAP may exist with different configurations since the molecule contains two centres of asymmetry. They are the first and the second carbon atoms of the propanediol chain. Two pairs of diastereoisomers, the erythro pair and the threo pair, may exist depending on the mutual position of the hydroxyl substituent at the first asymmetric carbon atom and the amino substituent at the second. It is the D(-) threo stereoisomer that possesses biological activity.[18] In contrast to the other stereoisomers, this is the only form capable in the prokaryotic cell of:

a. penetrating the bacterial cell wall

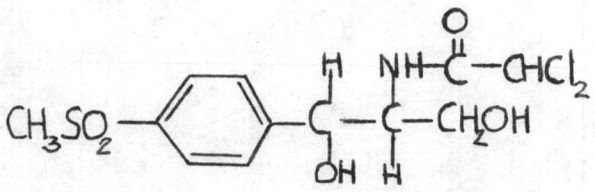
b. stereospecifically binding to the 50s ribosomal subunit and thus inhibiting peptidyltransferase activity

c. being acetylated by the acetyltransferase and reduced by the nitro-reductase of resistant bacteria

In eukaryotic cell CAP behaves as a substrate for several enzymes such as nitroreductase, amidase, dehalogenase and glucuronyl-transferase which are responsible for the biotransformation of this compound. The depression of hematopoiesis by CAP is due to inhibition of ferro-chetalase, mitochondrial protein synthesis and DNA synthesis.[18]

The chemical structure of CAP and a method for its synthesis were elucidated by Parke Davis Laboratories which distribute the drug as "Chloromycetin".[15] The ease by which CAP can be synthesized has led to the preparation of number of CAP analogues.

2.1 Thiamphenicol (TAP)



= Structure of Thiamphenicol

One of the well known analogue in which the p-nitro group on the benzene ring is replaced by a methyl-sulphonyl group. In fact, there are many analogues other than TAP which is synthesized by modifying the p-nitro group, but these analogues are invariably less active than CAP. Their value was limited to the in vitro determination of bacteriostatic activity.(18)

TAP is more soluble and more stable in solution than CAP. Its antimicrobial spectrum is similar to that of CAP. In therapeutic doses TAP, unlike CAP, causes haemopoietic toxicity. However, it has been claimed that TAP-induced bone marrow damage, though severe, is always reversible. In addition irreversible aplastic anemia (APA) has never been reported with the use of this drug.(17,18,19,20,21) These studies suggested the involvement of the p-nitro group of CAP in APA. In contrast with CAP, TAP is not conjugated with glucuronic acid in the liver to any extent, and is excreted in the urine as active unchanged form.(15,18)

2.2 Fluramphenicol, SCH 24893, SCH 25298

By oxidation of the dichloroacetyl part, some derivatives can be obtained in which

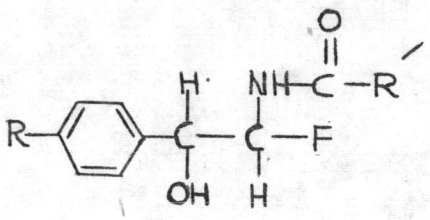
a. the two chlorine atoms of dichloroacetyl group are replaced by fluorine atoms.

b. the hydrogen atom is replaced by another halogen atom.

The above structural changes has been confirmed by an extension of <u>in vitro</u> study in which trifluoroacetyl derivative of CAP, fluramphenicol, unlike CAP, does not covalently bond to protein macromolecules when incubated with microsomal system.[45]

Other derivatives have been synthesized by substituting the hydroxyl group at position 3 with a fluorine atom. They are SCH 24893, SCH 25298.[22,23]

The structures of these three derivatives are as follows:



	R	R'
SCH 24893	$-NO_2$	$-CHCl_2$
SCH 25298	$-SO_2CH_3$	$-CHCl_2$
SCH 25393 (Fluramphenicol)	$-SO_2CH_3$	$-CHF_2$

These three derivatives are active against certain organism resistant to CAP and TAP. The therapeutic benefits of such compounds are not clear in view of the development of relatively non-toxic broad spectrum cephalosporin. However, it is possible that these agents could be useful in veterinary medicine.[22,23]

2.3 Another series of second generation derivative of CAP is characterized by changes in the alcoholic group at carbon atom 3 on the propanediol side chain. Primary hydroxyl group of CAP and TAP can be substituted with a fluorine atom, resulting in derivative that not only retains the bacteriostatic activity of CAP and TAP against sensitive bacteria, but also had full bacteriostatic activity against acetylated CAP and TAP-resistant bacteria.[18] These new compounds have only been recently identified, and much still remains to be learned before their chemotherapeutic

potential completely understood.[18]

Mode of action

The basic mechanism underlying the chemotherapeutic efficacy of CAP is the inhibition of bacterial protein synthesis which is dependent on its ability to bind to the 50s subunit of 70s ribosome. It appears that CAP inhibits the transpeptidation reaction which occurs at acceptor site.[26,28] Because its configuration is similar to that of uridine-5-phosphate, CAP competes with the uridine nucleotides, previously occupying the ribosome receptor, and thus interferes with the attachment of the m-RNA to the ribonucleoprotein activating.[27]

In bacterial cells which have been exposed to CAP for a short time protein synthesis may resume when the drug is removed. This explains the bacteriostatic action which this drug has against certain bacteria.[29] With more prolonged exposure, CAP has additional effect on bacteria resulting in extrusion of cellular macromolecules, cells lysis and degradation of ribosomes, these effects eventually lead to cell death. This bactericidal effect is dependent on the growth phase of the bacterial cells.

It has been suggested that the haemopoietic toxicity of CAP is due to the drug inhibition of human cell protein synthesis.[30] Immature or proliferating erythrocytes are much more susceptable to CAP than other mammalian cells. The mechanism by which CAP inhibits protein synthesis in mammalian cells is probably different from that operating in bacteria. Mammalian cells contain 80s ribosomes, and the protein synthesis in these structures appears to be unaffected by CAP. However, the mitochondria of human and other mammalian cells (which contain 70s particles) are also capable of independent protein synthesis. Martelo et al (1969)[31]

demonstrated that therapeutic concentrations of CAP inhibited protein synthesis by human and rabbit bone marrow mitochondria. Furthermore, Yunis et al (1970)[7] observed mitochondrial damage by electron microscopy in bone marrow cells obtained from patients treated with CAP. But there are also some evidences suggesting that CAP only acts on human mitochondria in experimental situations, as normal intact mitochondrial membranes are impermeable to this drug.[26] Nevertheless the effect of CAP on mitochondria appears to explain the dose-related haemopoietic toxicity of the drug, but the pathogenesis of the rare aplastic anemia remains unknown.[32,33,34]

Antibacterial activity and therapeutic uses

CAP is usually classified as bacteriostatic agent because in vitro condition this drug usually arrests the multiplication of bacteria, but does not reduce their number.[15] However, CAP in high concentrations may be "cidal" to some organisms, and it is even "cidal" in relatively low concentration to H. influenzae and Neisseria meningitidis.[1,24]

Although many species are susceptible to CAP, the use of this antibiotic should be restricted to certain serious infection or conditions.[1]

Diseases in which CAP is a first choice antibiotic are:

- Typhoid fever and other invasive salmonella infections
- Haemophilus influenzae systemic infections
- Anaerobic infections:

  Brain abscess

  Intra-abdominal abscess and bowel perforation
- Ventriculitis and meningitis
- Bacterial ophthalmitis

- Chronic granulomatous disease
- Rickettsial infection

Factors that favor the use of CAP are determined by the following characteristics of the drug:

1. CAP diffuses well into cell body fluid, therefore it is useful in meningitis, ventriculitis, and bacterial ophthalmitis.

2. CAP penetrates leucocytes and tissues, thus it is effective in typhoid fever and chronic granulomatous disease of childhood.

3. The diffusion of CAP into central nervous system tissue is superior to any other antibiotic because of its high lipid solubility. This fact, and the knowledge that anaerobic bacteria are almost universally present in brain abscesses, make CAP an ideal antibiotic to treat intracranial infections.

Resistance to CAP is primarily due to the presence of an inactivating enzyme, CAP acetyltransferase. The production of this enzyme is plasmid (R-factor) mediated.[21] Whereas other bacterial enzymes may modify CAP chemically, such enzymes do not seem responsible for resistance. Nonenzymatic resistance (permeability barrier to the antibiotic or step-wise mutation in the bacterial 50s ribosome) is rare.[1]

It is possible that aplasia in man may be caused by cell-mediated immune mechanisms, alternatively the stem cell may be directly damaged by the drug, and other mechanisms cannot be excluded.[35] Besides being an antibacterial, CAP may also be useful as an immunosuppresive drug for the treatment of diseases such as lupus glomerulonephritis or even for the prevention of homograft rejection.[15] Its use as tumor inhibitor has also

been reported.[36] This drug may be less toxic than other immunosuppressant because it inhibits antibody synthesis by different mechanism and does not cause cellular necrosis.[30]

Pharmacokinetics

1. Absorption

The ester of CAP are biologically inactive and must be hydrolyzed following administration to release the active compound.[1] The solubility characteristics of the drug result in its rapid and efficient absorption from the gastrointestinal tract when it is administered orally as the crystalline form (in capsules).[1] CAP palmitate is readily hydrolyzed in the proximal small bowel by pancreatic lipases, thereby releasing free CAP which is then absorbed as the active compound.[1] CAP succinate is partially hydrolyzed in vivo following intravenous administration. The mechanism by which hydrolysis occurs is not clear. But CAP glycinate, following intravenous or intramuscular administration, is rapidly and completely hydrolyzed. This compound gives the same pharmacological effects as a corresponding amount of free CAP.[37]

2. Distribution

CAP diffuses into many body tissues and readily penetrates into body fluid and ascitic fluids. It also capable of crossing the placenta.[15] Concentration of the drug in the ascitic fluid of patients with bacterial peritonitis usually exceeds half of the serum level at that time.[15,38] Unlike many other antibiotics, it penetrates well into all parts of the eye, and into the CSF even in the absence of miningitis. The CSF concentration may vary from 50 to 66.5 per cent of the serum level.[15,39]

CAP is protein bound to some extent, and as with other antibiotics

this binding is readily reversible. Some authors have found that the drug is 50 to 60 per cent protein bound[15] while others give a lower figure of 25 per cent.[41] Serum protein binding of CAP is lower in cirrhotic patients than in normal adults. Low binding of CAP was also found in the serum of premature neonates.[41]

3. Serum levels

Following intravenous administration of CAP succinate, the peak serum level is reached at an average of 45 minutes (30-60 minutes).[5] But after intramuscular dose, the blood levels reaches its peak about 2 hours,[15] which is about the same as for orally administered CAP base. With intramuscular CAP glycinate, the peak level is approximately the same as that attained after a similar succinate dose given intravenously, and reaches its peak within 45 to 60 minutes.[37] Serum concentrations between 10 and 20 mcg/ml are generally regarded as effective against susceptible pathogens. Therefore, optimal doses should result in serum concentration of this range.[1,3]

Numerous methods have been developed for measuring CAP in serum. The routine clinical utilization of any of those methods is limited by one or more shortenings as will be described below.

a. Colorimetric assay[70]

The basis of the method is the reduction of nitro group in CAP molecule to amino group, then diazotised and couple to a chemical which will form colored complex. However, it must be kept in mind that other drugs, such as sulfonamides, also contain free amino group which can react in a similar manner and given spurious result.

b. Radioenzymatic assay[12]

The assay is based on the enzymatic acetylation of CAP catalyzed

by R-factor-mediated enzyme. [^{14}C] acetyl coenzyme A serves as the donor of the labeled acetyl group; and the product, [^{14}C] acetoxychloramphenicol, is separated from the labeled precusor by extraction with benzene. The product is then quantitated with liquid scintillation counter.

This method offers sensitivity, specificity, precision, accurary, economy and ease of operation. But the method may prove unpractical in many clinical laboratories because the lack of commercial acetyl transferase enzyme and the need for associated accessory equipments.

c. High-Performance Liquid Chromatographic assay (HPLC)[13]

This method is rapid, accurate, precise and highly sensitive (less than 25 mcl of serum is required). Serum is first treated to precipitate proteins. CAP is then eluted with a mobile phase and the drug is measured at dual-wavelength. Each analysis requires less than 15 minutes. The HPLC will detect CAP in concentration as low as 0.5 mcg/1. But the major disadvantage is that this method measures the combination of both active CAP and its metabolites, which renders the determination of the exact amount of active CAP in the specimen rather difficult. Moreover, due to the high cost of equipment and complex analytical procedures involved, this method may be unpractical in many laboratories.

d. Gas Chromatographic assay.[13]

The general principle is the same as HPLC assay except the mobile phase is different. This method also shares similar problems with that of HPLC as it detects both CAP and metabolites simultaneously.

e. Microbiological assay[70]

This method is based on the ability of the drug to inhibit the growth of tested-micro-organism in agar diffusion plate. Problems usually

encountered with the assay are that a large serum sample required, lengthy analysis, and lack of specificity resulting from interference of some groups of antimicrobial agents in CAP inhibition zone. But this interference can be eliminated by prior enzymatic degradation of the interfering antibiotics.

This method is suggested to be the basis of all CAP assay because it is very useful for determining active CAP in serum and it generally used as a standard reference for existing or new assay methods.

4. Metabolism

This mechanism by which CAP succinate is hydrolyzed in vivo is poorly understood. But whatever the mechanism the hydrolysis is quite variable and unpredictable leading to the persistence of intact ester in the serum of some patients up to six hours following a single dose.(2) In infants during the first month of life, the hydrolysis of succinate ester proceeds less readily than older infants and children.(1) Thus, the metabolism of CAP will also vary widely among patients due to the variation in hydrolysis of succinate ester.(1)

After the ester is hydrolyzed, the remained active CAP in serum will be metabolized by many microsomal enzymes. There are at least three functional groups in the CAP molecule that are major determinants of its metabolic profile; they are p-nitrobenzene, dichloroacetyl radical and primary alcoholic group at third carbon atom of the propanediol chain. The end products of metabolism can be divided into 2 important groups:

4.1 Major metabolite is glucuronide conjugation. Active CAP is rapidly conjugated with glucuronic acid by specific enzyme in the liver called glucuronyl transferase. The product is CAP monoglucuronide which inactive and soluble in water. This reaction takes place in primary alcoholic

group at carbon 3 of propanediol chain.

4.2 Minor metabolites

P-Nitrophenyl group undergoes stepwise reduction to amino group. At present it is generally believed that this metabolite is produced mainly by bacterial nitroreductase of intestinal flora rather than by hepatic nitroreductase.[18]

Dichloroacetyl chain is oxidised at microsomal level by cytochrome P-450 monooxygenase and gives rise to at least two highly reactive radicals capable of binding covalently with the SH and NH_2 group respectively.[18] Thus the formation of the partially dehalogenated derivative occurs. Some studies indicate that these both derivatives are then incorporated into tissue proteins and may be involved in the toxic effect such as aplastic anemia.[43]

5. Elimination and Excretion

5.1 CAP is eliminated mainly in urine as:

a. Unchanged ester. This occurs only with the succinate ester. The succinate ester is subjected to renal elimination because of its high water solubility and dissociation as a weak acid. In 15 children receiving intravenous CAP, an average of 33 per cent, with a range of 6-80 per cent, of the administered dose was recovered in the urine as unhydrolyzed ester.[2] The renal clearance of CAP succinate in these patients was four times their concomitant creatinine clearance, indicating the ester is eliminated by active secretion from renal tubules rather than by glomerular filtration.

b. CAP monoglucuronide. This inactive metabolite is water soluble. Thus, it is excreted by both glomerular filtration and tubular

secretion in the kidney.[8] Eighty-five to ninety per cent of CAP is excreted as the glucuronide.

c. Active CAP is excreted only by glomerular filtration. Although most administered CAP is excreted in the form of inactive metabolites, 10 to 15 per cent of active drug in urine are still sufficiently high to be effective for treatment of urinary infection. Urine levels exceeding 200 mcg/ml of the active drug have been found following a single dose of 1.5 gm.[15] However much less CAP is excreted in the urine of patients with renal failure and consequently the urine CAP levels from some of these patients may be too low to exert antibacterial action.[44]

d. Minor metabolites. Only small amount of them are excreted in urine.

5.2 Minute quantity of CAP (2-3% of the administered dose) is excreted in bile, mostly in the inactive form. The concentration of active CAP in bile is usually lower than the concurrent level in the serum.[15]

5.3 About 1% of an orally administered dose of CAP is excreted in the feces, largely as the inactive product. It probably reaches the intestinal tract via the bile.

The variation in the rate of <u>in vivo</u> hydrolysis and variable renal elimination of CAP succinate markedly influence the serum concentration of active CAP which are achieved following intravenous administration. While succinate ester persists in the body, it serves as a "prodrug" reservoir continually releasing active CAP. This results in lower and later peak levels of the drug and accounts, in part, for the wide variation in calculated apparent half-life. Furthermore in contrast to free CAP little, if any, of the succinate esters diffuses into CSF.[1] Impaired liver

function may potentially diminish the conjugation of CAP and increase the risk of toxicity if the dose is not reduced. On the contrary renal failure, which can cause accumulation of the glucuronide conjugate, not augment the risk of toxicity and the dosage adjustment rarely required.

In conclusion, evaluation of CAP pharmacokinetic parameters and dosage regimen in individual patients is rather unreliable. It appears that the best approach to therapy is frequent monitoring of serum CAP concentrations with empirical adjustment of dosage when indicated.[41]

Toxicity

In spite of the efficacy of CAP against a wide variety of micro-organisms, its clinical use is limited by potential lethal toxicity such as:

1. Bone marrow depression.

This is the most important adverse effect of CAP.[15] Two forms of bone marrow depression are best documented.

1.1 Aplastic anemia (APA)

It is unlikely that APA represents a drug hypersensitivity. Moreover, the incidence of this effect appears to be independent of the dose and thus raising the dose does not necessarily increase the incidence.[1,19] The toxicity occurs after therapy is discontinued rather than while receiving CAP. APA is frequently irreversible but not fatal in all cases and complete recovery has been observed after a period of 5 years.[21] Fortunately, this potentially lethal reaction is rare. The estimated incidence varies between 0.002 and 0.004 per cent,[46] the values considerbly less than the incidence of anaphylactic reaction to penicillin which is 0.02 to 0.11 per cent. All blood cell lines are affected; pancytopenia with hypocellular bone marrow

may occur.

The mechanism of idiosyncratic APA associated with CAP administration has bot been clarified mainly because of the lack of appropriate animals models for the study, as APA caused by CAP is limited to only a few species. Several groups of investigators have tried to explain this toxicity based on in vitro studies. In 1967, Holt[48] made the comment that CAP administered by parenteral route have not been documented to casue APA. He hypothetized that a minority of patients possess special intestinal Enterobacteriaceae which degrade CAP, and the byproducts of this action, after absorption, is responsible for bone marrow toxicity. This hypothesis however has not been confirmed. Krishna et al[19] observed that interference with microbial flora by CAP may be in part responsible for the bone marrow aplasia induced in cattles, and this effect was markedly reduced by co-administration of mixture of B complex vitamins including folic acid and vitamin B_{12}. Nonetheless there is still not enough valid scientific data to support the notion that the occurence of APA is related to the route of administration.[15,47] Besides, it has been reported that APA also occurred even after administration of CAP eyedrops.[49]

Genetic factors appear to play a role, since suppression of DNA synthesis which induced APA following drug therapy is known to in identical twins.[50] Dameshek[51] proposed that there may be a genitically determined defect in the bone marrow cells of some individuals, rendering them particularly susceptible to CAP-induced bone marrow aplasia.

Viral hepatitis has been implicated as an important cause of APA. It is possible that CAP and the hepatits virus(es) may have additive toxic effects on the bone marrow.[15] Thus the risk of APA may greatly increase if

the drug is used during viral hepatitis.

Krishna et al[20] postulated that the free radical, formed by reduction of CAP in the immature bone marrow cells and its subsequent reaction leading to superoxide anion in the bone marrow, might play an important role in the toxicity of CAP. They proposed that the radical produced by reduction of nitroso CAP might not be as reactive as nitro anion radical mainly because of its low reactively towards oxygen and its stability. But Yunis[20] reported that hydroxylamine derivative or nitro group reduction might cause bone marrow toxicity in vitro. More specifically, it was suggested that this intermediate could act as a hapten by binding to some protein components of bone marrow cells. The antigen thus produced might trigger antibody production by the host, and subsequent antigen-antibody reaction resulting in bone marrow damage could take place.[20]

Races may also determine the risk of APA, for several reports show that APA occurs mostly in anglo-saxon rather than others races.[15]

However, all the evidences involved in CAP-induced APA remains to be further investigated.

1.2 Suppression of erythropoiesis

It predictably occurs if excessive serum concentrations of CAP are maintained for a sufficient length of time.[52] This toxic effect is dose-related and usually reversible. The ability of CAP to reversibly bind to 70s ribosome of mammalian mitochondria at concentration as low as 10 mcg/ml, thereby inhibiting mitochondrial protein synthesis, may be responsible to this dose-dependent bone marrow toxicity. Suppression of

bone marrow tends to occur with increasing frequency when peak CAP concentrations consistently exceed 25 mcg/ml or concentration at 6 hours after a dose is above 15 mcg/ml. Overt signs of bone marrow toxicity usually are not evident until 4 to 5 days initiating therapy. Early signs are:

a. arrest of erythropoiesis, increase free erythrocytes protophorphyrin, and eventually a decrease in erythrocyte count.

b. low reticulocyte count

c. reduced iron ultilization for haemoglobin synthesis, thus leading to elevation of serum iron.(53)

d. thrombocytopenia and neutropenia may occur within 2-3 weeks of therapy.

e. the platelet count may fall after 2-3 weeks of therapy

Recovery from these untoward effects is generally completed within 7 to 10 days after therapy has been stopped. The risk of dose-related bone marrow toxicity can be minimized by maintaining the serum concentration below 25 mcg/ml and limiting the duration of therapy to the minimum required for adequate treatment.(1)

Abundant evidence has shown that reversible bone marrow suppression by CAP is a consequence of mitochondrial injury, which is reversible upon removal of the drug. In addition, CAP causes <u>in vivo</u> suppression of heme synthetase accompanied by a block in the last step of heme synthesis. Other important mitochondrial membrane proteins are also involved such as cytochrome a and a_3, ultimately leading to depressed mitochondrial respiration, compromised cellular synthetic processes and cessation of cellular proliferation as can be shown in several systems including bone marrow culture.(20)

The mechanism by which nitroso CAP exerts its toxicity remains uncertain. The effects of nitroso CAP on the cell cycle of cultured chloroma cells suggest that nitroso CAP interacts with DNA itself, a mechanism thought to underly the carcinogenic property of nitroso compounds. Some authors consider that the division of CAP-induced blood dyscrasias into two separate categories(idiosyncratic APA and suppression of erythropoiesis) is not always clear cut and that a common pathogenic mechanism may be involved.[15] The occurence of both APA and leukemia following CAP may be a consequence of mutational changes induced by the nitroso metabolite.[20]

In summary, patients receiving CAP should have reticulocyte counts, white blood cell counts, platelet counts, and hemoglobin determinations performed at the onset of therapy. These blood studies should be performed at the beginning of CAP therapy and then approximately every three days if therapy is continued beyond one week.

2. Other hemopoietic side effects.

CAP prevents the expected reticulocyte response in patients with pernicious anemia treated with vitamin B_{12}. Similarly it prevents the expected response to iron in patients with iron deficiency anemia.[15] Hemolytic anemia develops in patients who are glucose-6-phosphate dehydrogenase deficient in conjunction with other factors such as infection, and in patient with a deficiency of erythrocyte gluthathione peroxidase.[54]

3. Gray syndrome

This is a type of circulatory collapse, which can occur in premature and newborn infants receiving large doses of CAP.[2,55] Many case reports involved infants who were receiving CAP in the doses of 100

to 200 mg/ kg/day and had serum concentrations between 70 mcg/ml and 250 mcg/ml. These serum levels are ten fold more than the concentrations necessary to treat susceptible infections.[12] Symptoms in affected babies usually develope within 60 to 72 hours after initiation of CAP therapy and consist of abdominal distention, vomiting, pallor, cyanosis and circulatory collapse which usually resulted in death within a few hours.[1,15] The term "gray syndrome" was coined from the appearance of these children. It is now known that babies of less than 4 weeks old have a diminished capacity for both conjugating and excreting CAP. A similar syndrome has also been described in old children and adults who have accidentally received CAP overdoses.[56] This toxic effect does not occur when such excessively high concentrations are avoided or when serum CAP levels do not rise above 20 mcg/ml.

As CAP readily crosses placenta, it should be used with caution in late pregnancy or during labour because of the risk of this toxic effect in the newborn. The use of CAP should at best be avoided by nursing mother since the drug is excreted into human milk.[15]

The mechanism of cardiovascular collapse is not well understood. However, mention of cardiac muscle abnormalities had been made in three cases reported by Sutherland.[58] Left ventricular dysfunction may be one of the important factors leading to cardiovascular collapse in infant with toxic concentration of CAP.[59] This toxic syndrome may be related to the ability of CAP to disrupt energy metabolism at the cellular level. CAP has been shown to inhibit mitochondrial electron transport and reduce oxygen consumption at concentration in excess of 63 mcg/ml.[1,18]

4. Optic neuritis

This complication has been described in a small number of patients treated with CAP who developed optic atrophy and blindness.[59,60] Most of these patients are children receiving prolonged CAP treatment for pulmonary infection. Blindness may occur without recognizable fundal changes. Vision may be partly restored after termination of the drug, but this is rather uncommon. Large doses of the B vitamins have been used to treat this condition. Peripheral neuritis has also been described in association with optic neuritis.[61]

5. Gastro-intestinal side effects

Nausea, vomiting and diarrhea occasionally occur, but these are much less frequent than with tetracycline. Pseudomembranous colitis may occur, but this is rare. Glossitis and stomatitis, sometimes associated with thrush, may also be uncountered.[15]

6. Allergy and hypersensitivity reactions.

These are very rare; but contact dermatitis, rashes, drug fever and even occasional anaphylaxis and angioneurotic edema have been reported.[15]

7. Bleeding due to increased prothrombin time

This may occur with prolonged oral administration of the drug. Decreased vitamin K synthesis results from a reduction of intestinal bacteria, and this effect can be rapidly corrected by administration of parenteral vitamin K.[62]

8. Ototoxicity

Hearing loss has been noted in a few children with H. influenzae meningitis who have been treated by CAP. This is most certainly a sequel of their meningitis and not a drug toxicity.[15] However, animal experiments

have shown that CAP ear drops can cause deafness, especially when the preparations contain more than 5% CAP and that they are instilled in the middle ear cavity.(63)

9. Encephalitis

These are very rare and reversible.(16)

Drug interactions

CAP is always prescribed concomitantly with other drugs such as:

1. Paracetamol

CAP is often given with an antipyretic such as paracetamol. When they are given concomitantly, the elimination of CAP is reduced and the half life of CAP increases to 15 hours. Ideally, CAP and paracetamol should not be prescribed together, but if they are either the dose of CAP must reduced or serum CAP concentrations monitored.(64)

2. Phenytoin, tolbutamide, chlorpropamide and dicoumarol

CAP inhibits the activity of certain liver enzymes, and in doing so it interferes with the biotransformation of these four drugs.(65)

3. Phenytoin and Phenobarbital

Both drugs can induce hepatic microsomal enzymes and their use with CAP may increase the rate of CAP metabolism.(66) Although a previous report(65) indicated that CAP inhibited phenytoin metabolism, later study(66) suggested that phenytoin enhanced CAP metabolism. Other possible explanations for the metabolism changes may include increased hepatic blood flow and CAP induction of its own metabolism.

From all points of view, it ellustrates that monitoring of CAP serum concentration should be performed during the CAP therapy, especially when concomitant drugs, induced hepatic microsomal enzymes, are used. Since the interactions may be particularly dangerous in some occasions such as when treating the central nervous systems infection, which CAP concentration in CSF may be only 30 to 50% of serum levels.(67)