CHAPTER II

REVIEW OF LLTERATURES

The development of the tetracycline antibiotics was the result of a systematic screening of soil specimens collected from many parts of the world for antibiotic-producing micro-organisms (2). The first tetracycline to be introduced was chlortetracycline in 1948. Presently, six tetracycline analogues are marketed in the United States. Tetracycline, oxytetracycline [Terramycin^{R}], and demeclocycline [Declomycin^{R}] are naturally derived compounds from various species of Streptomyces. Methacycline [Rondomycin^{R}] and doxycycline [Vibramycin^{R}] are derived semisynthetically from oxytetracycline, and minocycline [Minocin^{R}] is prepared by chemical modification of tetracycline (7).

The tetracyclines are broad spectrum antimicrobial agents. In general, patterns of microbial susceptibility and resistance to the tetracyclines are similar, but there are some differences in the degree of activity among the various analogues. The newer tetracyclines, minocycline and doxycycline, are more active than the parent compound against some organisms (7).

These antibiotics are usually subdivided into

1. Short-acting : tetracycline, oxytetracycline
2. Intermediate-acting : demeclocycline, methacycline
3. Long-acting : doxycycline, minocycline.

Review of Doxycycline

1. Physicochemical Properties (8, 9, 10, 11)

The molecule of doxycycline shares a similar skeleton with all tetracyclines, 6-demethyl 6-deoxytetracycline (3). It's chemical name is 4-(Dimethylamino)- 1, 4, 4a, 5, 5a, 6, 11, 12a -octahydro-3, 5, 10, 12, 12a -pentahydroxy -6 methyl -1, 11-dioxo -2-naphthacene-carbaoxamide monohydrate.

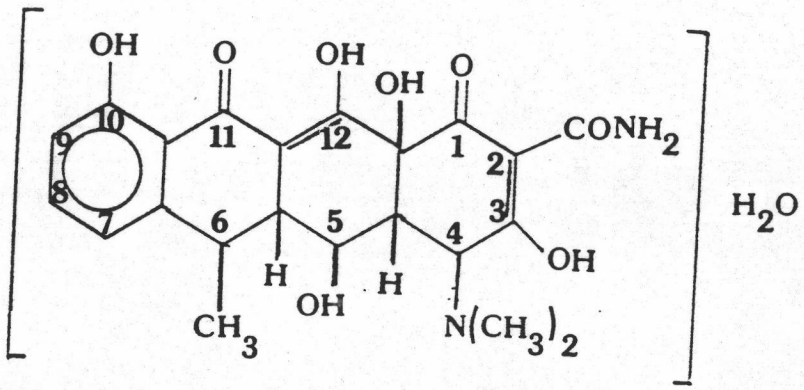

Figure 1. Structural formula of doxycycline.

Synonym	:	Doxycycline monohydrate
Empirical formula	:	$C_{22}H_{24}N_2O_8, H_2O$
Molecular Weight	:	462.5
Description	:	A yellow crystalline powder.
Solubility	:	Very slightly soluble in water, sparingly soluble in alcohol; practically insoluble in chloroform and ether; freely soluble in dilute acids and alkali hydroxide.
Dissociation constant [pKa]	:	3.5, 7.7, and 9.5 (20°C)

Partition coefficient	:	log P (octanol /pH 7.5), -0.2
pH	:	Between 5.0 and 6.5, in an aqeous suspension containing 10 mg per ml
Packaging and storage	:	Preserve in tight, light-resistant containers.
Stability	:	Doxycycline is probably the most stable product of the group of tetracyclines. Since the molecule carries a hydroxyl group at C-5, it is more stable towards C-4 epimerisation. This is probably due to hydrogen bonding of hydroxyl with the C-4 dimethylamino group. The absence of a hydroxyl at C-6 excludes the possibility of acid degradation to the corresponding anhydro derivative (11).
Melting point	:	Chars without melting at about 201° C (12).

2. Mechanism of Action

The site of action of doxycycline is the bacterial ribosome (2). Doxycycline appears to inhibit protein synthesis in susceptible organisms mainly by reversible binding to 30S ribosomal subunits, thereby inhibiting the formation of the tRNA - aminoacyl - mRNA - ribosome complex and consequently translation (3,13). Penetration of doxycycline into susceptible organisms is biphasic; adsorption to plasma membranes is followed by active transport through the inner cytoplasmic membrane . The second step is dependent of the extent of adsorption (3).

Doxycycline also impairs protein synthesis in mammalian cells at high concentrations; however, the host cells lack the active transport system found in bacteria (2).

At clinically tolerated doses, doxycycline is bacteriostatic for most micro-organisms and bactericidal for some. At higher concentration, doxycycline may have a bactericidal effect. A critical serum concentration of 4 µg/ml may be accepted for doxycycline due to excellent tissue penetration (3).

3. Antimicrobial Spectrum

As for its tetracycline congeners, doxycycline exhibits a wide range of activity against both aerobic and anaerobic gram-positive and gram-negative organisms, Rickettsiae, Chlamydiae, Mycoplasmae, spirochetes, and protozoa (3).

3.1 Gram - negative - aerobic organisms

Activity against Haemophilus species, Neisseria species, Brucella species, Vibrio species, Moraxella species, and Pasteurella is excellent (3,14,15). Klebsiella species including Klebsiella pneumoniae, Escherichia coli, Salmonella species, Enterobacter species, Serratia, Shigella species are variably susceptible to doxycycline. Proteus mirabilis and Pseudomonas aeruginosa are intrinsically resistant to the tetracyclines (3).

3.2 Gram - positive aerobic bacteria

Susceptibility to doxycycline of Staphylococcus species, Corynebacterium species, and Bacillus species is excellent while the sensitivity of Streptococcus species is intermediate. Sensitivities depend on the class within a species, on the geographic area where the organism has been isolated and the freqeuncy of doxycycline used (14,16).

3.3 Anaerobic bacteria

Activity against Fusobacterium, Actinomyces and Propionibacterium species is excellent. Bacteroides fragilis, Clostridium, Lactobacillus, and Peptococcus species demonstrate a moderate sensitivity to doxycycline (17).

3.4 Other micro-organisms

Spirochetes, Mycoplasma species, Rickettsia species (18) Chlamydia trachomatis, Ureplasma urealyticum, and Anaplasma species demonstrate a good susceptibility to doxycycline (3,13).

3.5 Resistance

Several species of bacteria have become increasingly resistant to the tetracyclines. Many Enterobacteriaceae and most P. aeruginosa are resistant. Many strains of staphylococci, streptococci, and Bacteroides are no longer susceptible. Even some strains of Pneumococci and N. gonorrhoeae have become resistant to the tetracyclines (7).

4. Pharmacokinetics

4.1 Absorption

The pharmacokinetic behavior of doxycycline in humans has been studied by several investigators (19-29). Absorption of orally administered doxycycline in the usual single dose of 100 mg to 200 mg is higher than 80%, the mean values being close to 95%, and there is no significant difference between the various salts.

Absorption is rapid, traces of the antibiotic can be detected in the blood sample 15 minutes after administration (19). Following oral administration of capsules containing doxycycline hyclate in fasting adults with normal renal function, peak serum concentrations of doxycycline are attained within 1.5-4 hours and average 1.5 - 2.1 µg/ml following a single 100-mg dose and 2.6-3 µg/ml following a single 200-mg dose. GI absorption of doxycycline salts may be reduced up to 20% by food and / or milk; however, the effect is not usually clinically important (23). Of the currently available tetracyclines, doxycycline has the least affinity for calcium ions (12).

The persistence of serum doxycycline level has been explained by Gibaldi (20) to be due to slow absorption compounded by enterohepatic cycling. This may be due to the high affinity of doxycycline to the bile. A secondary plasma peak indicating reabsorption was noticed regularly at 8 hour after drug intake (20).

4.2 Distribution

Because of its lipophilicity, doxycycline shows excellent tissue distribution (18). In mice, rats and men, the ratio of tissue concentration to blood concentration is greater than 1 in muscle, lung, heart, testicle, renal cortex and medulla, prostate, bladder, gallbladder, stomach, and intestine , but lower than 1 in fat, lymphnode and brain (3).

Plasma protein binding of doxycycline is estimated at between 80 and 90%. It thus exhibits the strongest protein binding of all the tetracyclines (19).

The volume of distribution of doxycycline at steady

state varies according to the authors between 52.6 and 134 litre and the volume of the central compartment is 22 litre (19).

4.3 Elimination

Doxycycline was considered by some workers to be metabolically inert because metabolites could not be detected (30). More recently; however, a metabolite of doxycycline has been detected in liver and kidney of laboratory animals and in human urine (27).

The excretion of doxycycline occurs by three major routes : the kidney, the liver and the gastrointestinal tract. More than 90% of labelled doxycycline was recovered in feces and urine of humans (3).

All the pharmacokinetic studies reported the elimination half lives ($t_{1/2}\ \beta$) for doxycycline varying between 15 and 25 hours, which is longer than other tetracyclines (19).

5. Dosage and Administration

Normally, doxycycline is manufactured as oral dosage form; it is available as the hydrochloride salt for capsules or tablets or as the calcium chelate or monohydrate for syrup or suspension (10). When oral therapy is not feasible, doxycycline hyclate or hydrochloride may be administered by slow IV infusion; however, oral therapy should replace IV therapy as soon as possible (12).

The usual oral dose of doxycycline for adults and children older than 8 years of age weighing more than 45 kg is 100 mg for every 12 hours on the first day of treatment followed by 100 mg

daily in 1 or 2 divided dose. For severe infections, these patients may receive 100 mg every 12 hours. The usual oral dose of doxycycline for children older than 8 years of age and weighing 45 kg or less is 4.4 mg/kg given in 2 divided doses on the first day of treatment followed by 2.2 mg/kg daily given in 1 or 2 divided doses. For severe infections, oral dose up to 4.4 mg/kg daily may be used in these children (12).

Doxycycline has been shown to prevent travelers'diarrhea caused by enterotoxigenic E.coli (7). Although the anti-infective agents for prophylaxis of travelers' diarrhea is generally discouraged, if doxycycline is used, the recommended adult oral dose of the drug is 100 mg once daily, the period of risk (for up to 2 weeks) beginning in the day of travel and continuing for 2 days after returning home.

Dosage in renal impairment

Unlike other currently available tetracycline derivatives, usual dosage of doxycycline may be used in patients with impaired renal function (12).

6. Adverse Effects

6.1 Gastrointestinal tract

All tetracyclines produce gastrointestinal irritation to a varying degree in some but not all individuals (2). Manifestation of gastrointestinal disturbances after administration of tetracyclines includes irritation of the stomach and upper intestine, and suprainfection which may result in bacterial toxin release and induce a severe

inflammation of the large intestine. In comparative studies in man, doxycycline was found less harmful to the fecal flora than tetracycline as judge by reduced appearance of resistant bacteria and less overgrowth of Candida, Staphylococcus, Streptococcus, Pseudomonas, Proteus and Klebsiella species (3). There have been a number of reports of oesophagitis ulceration associated with administration of doxycycline tablets or capsules. The acidity of tetracycline and doxycycline and the rate of dissolution and release of the drug have been linked to the toxic effect; the doxycycline monohydrate free base formulation has been found to be less toxic to the cat oesophageal mucosa than the hydrochloride and dispersible tablets are less likely than capsules to stick to oesophageal mucosa (10). Side effects on the lower bowel after oral administration of doxycycline have been infrequent in man (3).

6.2 Renal effect

Doxycycline has been reported to produce fewer renal side effect than do other tetracyclines; however , a possible association between this drug and the production of renal failure has been suggested (2). Unlike the other analogues, doxycycline is excreted by the gastrointestinal tract, under these circumstance., its half-life will remain unchanged and it will not accumulate in the serum of patients with renal insufficiency (7).

6.3 Hepatic effect

Hepatotoxicity, characterized histologically as fatty metamorphosis of the liver without necrosis or inflammatory reactions and sometimes associated with pancreatitis, has been reported rarely with tetracyclines (12). It is induced by high doses of tetracyclines

or accumulation of tetracyclines in renal failure (31). Doxycycline is less hepatotoxic than tetracycline in mice. Female mice are reported to be more susceptible than male mice (3).

6.4 Cardiovascular system

Rapidly IV injection of doxycycline at high doses (8 mg/kg) induced a pronounced decrease of blood pressure in 4 out of 10 anesthetized cats. The effect was absent at 4 mg/kg or when 8 mg/kg was administered over a period of 2 min. In another study, 10 mg/kg dose administered to dogs, resulted in low ventricular and arterial pressures, and bradycardia. These effects were dose dependent and more pronounced at 30 mg/kg (3).

Slow IV administration of doxycycline does not cause local irritation in man. Hemostatis is not affected by therapeutic doses of doxycycline (3).

6.5 Bones and teeth

Tetracyclines are deposited in developing bones and teeth where they can chelate with calcium to form a tetracycline calcium orthophosphate complex (7). Doxycycline has a lower affinity for binding with calcium and it may cause less tooth discoloration than other tetracyclines (10,32). The extent of discoloration is dose dependent and occurs during odontogenesis of deciduous and permanent teeth.

6.6 Dermatologic effects

Photosensitivity, manifested as an exaggerated

sunburn reaction on sun-exposed areas of the body, has occurred with tetracyclines (12). Demeclocycline is more prone to induce phototoxic dermatitis than tetracycline. In vivo studies in man indicated that doxycycline is less phototoxic than demeclocycline (33). However, a large incidence of sunburns associated with paresthesia may occur after heavy sun exposure following doxycycline used (34). In vitro studies investigating the photohemolytic potency of tetracyclines, revealed that doxycycline and demeclocycline had more pronounced hemolytic properties than the other tetracyclines (35). The phototoxic reaction of doxycycline is provoked by long-wave and medium-wave ultraviolet radiation (36).

6.7 Thrombophlebitis

Of 46 patients with infusion phlebitis, doxycycline hydrochloride was implicated as the causative agent in 11 patients. The incidence of doxycycline-induced phlebitis was reduced when the drug was administered in 100-ml rather than 50-ml solutions. It was recommended that a dose of 100 mg should be administered with at least 200 to 250 ml of IV fluid over 1 to 2 hours (10).

7. Drug interactions

Metallic ions such as calcium, aluminium, magnesium, and iron, strongly diminish the bioavailability of tetracyclines after oral administration due to the formation of insoluble complexes. With the exception of iron, doxycycline absorption is less affected than the other tetracyclines by the presence of metallic ions and by food (3). Therefore unlike the older tetracyclines, doxycycline may be administered with a meal (3). In one study, oral ferrous sulfate reportedly decreased the serum half-life of a single IV dose of doxycycline as

the hyclate, presumably by interfering with intestinal reabsorption of the anti-infective (12). Concurrent oral ferrous sulfate administration lowered the serum concentration of doxycycline administered by mouth. This interaction could not be avoided completely by leaving a 3-hour interval between doses of the two drugs (10). In another study, concomitant administration of oral zinc sulfate impaired absorption of oral tetracycline hydrochloride but no effect on the absorption of oral doxycycline was reported (12).

The half-life of doxycycline may be decreased by concurrent administration of carbamazepine, phenytoin or barbiturates, which increase the hepatic metobolism of this antibiotic (7).

Antidiarrheal agents containing kaolin and pectin or bismuth subsalicylate reportedly impaired absorption of tetracyclines(12). The bismuth subsalicylate contained in a 60-ml dose of Pepto-Bismol significantly decreased the bioavailability of 200-mg oral doxycycline (37). Thus, the two agents should not be taken together to prevent travelers' diarrhea.

Short-term administration of alcohol has been shown to increase the elimination half-life of doxycycline, and the half-life of doxycycline has been reported to be reduced in recently abstinent alcoholic patients compared with healthy controls (38).

8. Laboratory Test Interferences

8.1 Test for urinary glucose

Although tetracyclines have reportedly caused false-positive results in urine glucose determination using the cupric

sulfate method [Benedict's reagent, Clinitest ®], this effect may have been caused by ascorbic acid which is included in parenteral preparation of tetracyclines. Tetracyclines also have reportedly caused false-negative results in urine glucose determinations using glucose oxidase reagent [Clinistix ®, Tes-Tape ®] (12).

8.2 Other laboratory tests

Doxycycline interferes with fluorometric determinations of urine catecholamines resulting in falsely increased values (12).