CHAPTER II

REVIEW OF LITERATURES

Norfloxacin

Physicochemical Properties

Norfloxacin (MK-0366, AM-715) is a new orally absorbed synthetic organic acid (ɣ pyridone β carboxylic acid) structurally related to nalidixic acid with much greater intrinsic antibacterial activity (22,31,34,36-42).

Structural formula:

Norfloxacin is a quinolone derivative, with the chemical name 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

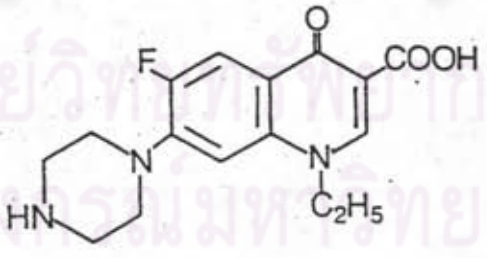

Figure 1. Structural Formula of Norfloxacin

Molecular formula: $C_{16}H_{18}O_3N_3F$

Molecular weight: 319.33

Melting point: 222.5 °C

Appearance: White odorless powder, bitter taste.

Hygroscopicity: Equilibrium at 14 % corresponding to 2.5 % H_2O at 60-70 % relative humidity.

Stability:

Norfloxacin substance has been shown to be stable for at least 3 years at room temperature. Exposure to sunlight will produce a colour change from white to yellow to brown respectively with loss of activity.

Mechanism of Action and Development of Resistance

Norfloxacin, as the other 4-quinolones, acts by inhibiting the DNA replication of bacterial cells by acting on DNA nicking-closing enzyme (responsible for DNA elongation) and on the subunit-A protein of the essential enzyme DNA gyrase (that supercoils the DNA molecule) (27,41,43-45). Norfloxacin, unlikes the other 4-quinolones, can bind to DNA when enzyme DNA gyrase is not associated with DNA (as in non-dividing bacteria)(46). The critical imbalance effect of the cellular metabolism is bactericidal. At the higher drug concentration, norfloxacin has been shown to inhibit RNA synthesis resulting in a bacteriostatic effect (44).

Quinolones still have a significant advantage over other commonly used agents in that resistance to quinolones is neither dependent on destruction of antibiotics by enzymes nor plasmids mediated (31,41,44). Additionally, these antibacterial agents often cause plasmid elimination from bacteria which harbour them and can block the transfer of plasmids (31).

Resistance to norfloxacin has so far been slow to develop clinically and the agent shows good activity against many multiresistance strains of bacteria (31). Nevertheless, resistance to norfloxacin can be induced experimentally (47-50).

Cross-resistance has been observed between norfloxacin and nalidixic acid as well as some of the newer nalidixic acid analogues (ciprofloxacin, enoxacin, cinoxacin and oxolinic acid) and in a few instance, some β-lactam antibiotics (31,47,48,51,52).

The mechanism(s) of resistance to norfloxacin and the other 4-quinolones are yet unknown. These mechanisms may be resulting from bacterial mutation associated with changes in outer membrane proteins (31,41,53) or the modification of the subunit-A protein of DNA gyrase (41, 51,54,55).

In Vitro Antimicrobial Activity

Activity Against Gastrointestinal Tract Pathogens

Norfloxacin is proved to be effective against a range of gram-negative organisms which commonly occur as gastrointestinal tract pathogens. Both Salmonella species and Shigella species are susceptible to norfloxacin, with minimum inhibitory concentration for 90 % of tested strains (MIC_{90}) values of 0.05 to 0.25 mg/L and 0.06 to < 0.5 mg/L, respectively (22,27,29,31,34,39,42,50,56-58). Yersinia enterocolitica (MIC_{90} : 0.03 to 0.09 mg/L) (22,27, 29,31,40,50,56-58), Aeromonas hydrophila (MIC_{90} : 0.125 mg/L) (29,31,34,50,56-58), Plesiomonas shigelloides (MIC_{90} : < 0.063 mg/L) (31,56), Vibrio parahaemolyticus (MIC_{90} : 0.06 mg/L) (22,27,31,56), and Vibrio cholerae (MIC_{90} : 0.015 mg/L) (22,27,31), are all easily inhibited by norfloxacin. Campylobacter, a gram-negative - microaerophilic organism, is inhibited by norfloxacin at MIC_{90} 0.25 to 4 mg/L (31,22,50,42,56-58).

Activity Against other Organisms

Norfloxacin is a broad-spectrum antibiotic with remarkable activity against most gram-negative bacteria. Most gram-negative pathogens including Escherichia coli, Klebsiella, Enterobacter, Proteus and Citrobacter species are susceptible to norfloxacinat at the concentrations of 2 mg/L or less (31,36-42,50,57). Acinetobacter, Providencia and Serratia species are slightly less

sensitive (MIC_{90} : < 1 to 32 mg/L) (31,36-39,41,50). 90 % of Pseudomonas aeruginosa isolates are inhibited by norfloxacin 1 to 2 mg/L (24,31,36-40,42,50). Haemophilus influenzae demonstrates marked sensitivity to norfloxacin (MIC_{90} : < 2 mg/L) (31,37,38,41,42,50), as do Neisseria gonorrhoeae and Neisseria meningitidis (31,37,41,42,50).

Staphylococci are susceptible to norfloxacin (MIC_{90} : 1 to 4 mg/L) but the Streptococci, including Enterococci, are more resistant (MIC_{90} : 2 to 16 mg/L) (24,31,37-42,57). Most strains of anaerobic bacteria are moderately sensitive to norfloxacin, while some are resistant (22-28). Although norfloxacin has no intrinsic antifungal activity there is some evidence of synergy with antifungal against Candida species (31).

Clinical Efficacy of Norfloxacin

Preclinical study in rats (23) and an open study by Lolekha (35) proclaim the use of norfloxacin in the treatment of acute diarrhoea. Norfloxacin 800 and 1200 mg daily are proved to be as effective as co-trimoxazole 320/1600 mg daily in the treatment of acute diarrhoea (29,33-34). Norfloxacin 400 mg daily is proved to be effective in prevention of travellers' diarrhoea (29,32).

Norfloxacin is also proved to be effective in the treatment of acute and chronic infections of genito-urinary, gonorrhoea, biliary tracts, and other infections (31,60). Due to its favorable antibacterial properties, there are

also the potential uses of norfloxacin in prophylactic and suppressive long term treatment in urinary tract conditions, urological surgery, colonic surgery and in the suppression of aerobic and facultative bowel flora in granulocytopenic patients (31,33,59).

Dosage and Administration (31)

In acute bacterial gastroenteritis the usual dosage is 400 mg twice daily for 5 days.

In the treatment of urinary tract infections, the usual adult dosage is 400 mg twice daily continued for 7 to 10 days. Three-day therapy has been shown to be effective in women with uncomplicated acute cystitis. In the treatment of chronic, relapsing urinary tract infection, the dosage of norfloxacin is 400 mg twice daily for up to 12 weeks; if adequate suppression is obtained within the first 4 weeks the dosage of norfloxacin may be reduced to 400 mg daily.

In patients with renal failure, whose creatinine clearance is less than 30 ml/min/1.73 mm^2, the recommended dosage is 400 mg daily.

In the prophylaxis of sepsis in profound neutropenia, the recommended dosage is 400 mg 3 times daily for the duration of profound neutropenia. However, data for recommending treatment beyond 8 weeks are presently not available.

The safety and efficacy of norfloxacin in children have not been established.

Adverse Reactions and Tolerability

Norfloxacin has been generally well tolerated (see Appendix A). Gastrointestinal disturbances are the most commonly adverse effects, occur in 2 to 4 % of the patients, nausea and vomiting are the most frequent symptoms noted. Central nervous system reactions are generally minimal but include lightheadedness, drowsiness, headache and dizziness (31).

The incidence of abnormal laboratory values is also low (see Appendix A).

The adverse reactions and laboratory test abnormalities are also not found to correlate with the dosage, number of doses or the duration of administration of norfloxacin (31).

Toxicology

Due to unpublished data from Astra, no signs of retinal and inner ear toxicity nor signs of mutagenicity nor signs of oncogenic effect nor evidence which would indicate any antigenicity has been found with norfloxacin in animal studies. Due to the finding of arthropathy in juvenile dogs and rabbits, norfloxacin should not be given to children. It can be concluded that norfloxacin can be given to man with sufficient safety.

Pharmacokinetics of Norfloxacin

Absorption and Serum Concentration

Following oral administration, absorption of norfloxacin is rapid but incomplete and slightly reduced in postprandial administration (31,61,62). Serum concentrations are fairly low (63). Mean maximum serum concentrations (C-max) after single oral dose of norfloxacin 200, 400, 800, 1200 and 1600 mg are achieved within 1-2 hours (t-max) (31,61,62,64). With increasing doses, C-max, the area under the serum concentration time curves (AUC) from 0-12 hours and the urinary recovery of unmetabolized drug all become progressively lower relative to the dose (62,64). In multiple dose administration there is no evidence of norfloxacin accumulation (31,62).

Bioavailability

Recovery of norfloxacin in stools after single oral 400 mg doses averaged 28 % over the ensuing 48 hours (30). As the drug is excreted in the bile to only a small extent, these results imply an oral bioavailability of approximately 70 % (31).

Distribution

Norfloxacin penetrates well into peripheral compartments. Concentrations in palatine tonsillar tissue, maxillary sinus mucosa, vaginal tissue, cervix tissue,

salpinges, ovaries, renal cortex and the gallbladder wall are only slightly lower than serum concentration. Concentrations higher than those in serum are found in both common duct and in gallbladder bile as well as in liver tissue and renal medulla. In amniotic fluid and umbilical serum, concentrations are lower than those in the mothers'serum, while no detectable concentration could be demonstrated in breast milk (31,61-63).

Unpublished data from Merck Sharp & Dohme suggest a protein binding level of 14 %.

Elimination

Norfloxacin is mainly eliminated by active renal tubular secretion and glomerular filtration (65). Approximately 30 % of an oral dose is excreted as unchanged norfloxacin in urine (31,61,62). Six metabolites, 1 or more of which are thought to be microbiologically active, have been found in urine but account for less than 10 % of an administrated dose (31).

The biliary secretion (1-2 %), is an insignificant route of elimination.

The elimination half-life ($t_{1/2}$), of norfloxacin in healthy subjects ranges from 3.5 to 6.5 hours (31).

Diarrhoea

Definition of Diarrhoea

The definition of diarrhoea in a clinical sense is an increase in frequency or increased fluidity of bowel movements in a given individual (66,67).

Classification of Diarrhoea

Diarrhoea can broadly classified into two groups: Acute diarrhoea with sudden onset and short duration, caused mostly by infections (bacteria, viruses or protozoa), and chronic diarrhoea, often with an insidious onset and a long duration. Chronic diarrhoea is often a functional symptoms, but may be a manifestation of serious illness. (68,69)

Acute Bacterial Diarrhoea

Etiologic Agents

The bacterial enteropathogens causing acute diarrhoea can be broadly divided into two groups: noninvasive and invasive bacteria (70).

Noninvasive bacteria causing diarrhoea are:

Vibrio cholerae	*Bacillus cereus*
Vibrio cholerae non-01	*Klebsiella* species
Enterotoxigenic *E.coli*	*Enterobacter* species
Aeromonas hydrophila	*Proteus* species
Clostridium perfringens	*Staphylococcus aureus*

Invasive bacteria causing diarrhoea are:

Shigella species	Campylobacter jejuni
Salmonella species	Enteroinvasive E.coli
Vibrio parahaemolyticus	Yersinia enterocolitica

Besides these pathogens, other pathogens such as Citrobacter species, Edwardsiella tarda and Serratia species are also the potential causes of diarrhoea.

Pathophysiology (69-77)

The enteric bacterial infections may also be classified by the anatomic section of the intestinal tract that is their chief target. Small bowel diarhoea and large bowel diarrhoea can easily be distinguished.

The noninvasive pathogens, in general, affect the proximal and mid-small bowel. These pathogens cause symptoms by mediating enterotoxin, liberated from multiplying but non-invasive pathogens. This enterotoxin binds to the intestinal epithelium and causes massive secretion of fluid and electrolytes to the bowel lumen with only few anatomic mucosal changes. Thus, small bowel bacterial diarrhoea is associated with cramping, bloating, periumbilical pain and large-volume watery, nonbloody stools unassociated with intestinal inflammation (no fecal leukocytes). Systemic symptoms, including headache, myalgias, chills and fever are minimal or absent.

The invasive pathogens are more characteristically pathogens in ileocolonic diarrhoea. These pathogens produce virulence factors that cause anatomic damage to the host cell membranes. Inflammatory responses result either from direct mucosal penetration with cell destruction (occasionally with septicemia) or from excessive and aberrant host immune responses. Large bowel diarrhoea is commonly accompanied by fever, other systemic symptoms, lower abdominal pain and tenesmus. Stools are usually of small volume with fecal leukocytes and often bloody or mucoid.

Besides making their main impact on the host by invading the intestinal epithelium, the invasive pathogens also possess the mechanism of fluid production. The precise mechanism of this fluid production is not known. This mechanism may due to the enterotoxins elaborated by these pathogens or due to the increase local synthesis of prostaglandins at the site of intense inflammatory reaction that resulting in fluid secretion into bowel lumen or may due to prevention of fluid reabsorption from the lumen by the injured epithelial surface.

Diagnosis

Diagnosis of acute bacterial diarrhoea is based on pathophysiology, signs and symptoms of diarrhoea. Specific diagnosis is based on careful examination of

diarrhoeal material for polymorphonuclear cells and bacteria and, if indicated, for parasites. This is best accomplished at sigmoidoscopy prior to preparation with a cleansing enema. Cotton swabs should not be used in making slides, as both polymorphonuclear cells and parasites would cling to cotton. Rectal biopsy may prove helpful. These studies should be performed prior to barium studies and treatment (67).

Treatment

The goals of therapy include 1) prevention of dehydration, 2) rehydration if necessary, 3) relief of symptoms, and 4) shortening the course of the illness and reduce the spread of infections if possible, i.e., with antimicrobial agents.

Principle of acute bacterial diarrhoea treatments are as follows:

1. Specific treatment
2. Supporting treatment
3. Symptomatic treatment

1. Specific Treatment

The advantages and disadvantages of antibiotics in the treatment of acute bacterial diarrhoea are described on page 2. In general, in mild disease (small volume diarrhoea, no chills or fever, no blood or pus in the stool), antibiotics should not be prescribed

unless a specific indication emerges from the bacteriology and parasitology laboratory. In patients who are severely ill, especially if they have blood or pus in the stool, antibiotic therapy is reasonable, pending the result of stool culture (78).

Some recommended antibiotics for the treatment of acute bacterial diarrhoea are shown in Appendix B.

2. Supporting Treatment

The most important aspect of therapy in acute diarrhoea, and in some patients with chronic diarrhoea, is prevention or correction of salt and water depletion. This can be done by oral ingestion of liquids and salty foods, oral glucose-saline solutions, or intravenous fluid therapy, as dictated by the clinical situation (6,7,78).

3. Symptomatic Treatment

Opiates and other antiperistaltic agents (Diphenoxylate, Loperamide) may be administered for brief, 1 to 2 day courses of 2 to 8 tablets or capsules per day to control frequent watery diarrhoea. These drugs should be avoided in patients with frank dysentery, high fever, or toxemia when artificial retention of toxins or enteropathogens in the gut lumen may worsen the symptoms (79).