CHAPTER 1

INTORDUCTION

General Introduction of Piperacillin

Few development in the history of medicine have had such a profound effect upon human life and society as the development of power to control infections due to microorganisms.

Discovery of penicillin and many antibacterial agents have become a substantial part of medical care. These antibiotic included penicillin and related compounds, aminoglycosides, macrolide group, tetracycline group, chloramphenicals, peptide antibiotics and unclassified antibiotics (Kucer *et al.*, 1979).

Penicillin is the interesting drug group. Penicillin G (benzyl penicillin) was the first natural penicillin to be introduced for therapeutic purposes, followed by Penicillin V (phenoxy methyl penicillin). Later, the scientists were able to isolate the basic penicillin nucleus (6-amino-penicillianic acid) from penicillin G. Semi-synthetic penicillins were prepared by synthesized from the basic penicillin nucleus or by chemical modification of a nutural penicillin G (Grayson, 1982; Weinstein, 1975).

Both Penicillin (Penicillin G and V) covered gram positive bacteria included *Streptococcus* and *Staphyllococcus spp.* They were ineffective against gram negative bacteria such as *E. coli*, *Klebsiella*

spp. and *Salmonella spp.* excepted *gonococci* and *meningococci* (Kucer, 1979).

For the years, there have been the improper use of antibiotics on a large scale. Bacterial resistance occured rapidly, such in the case of *Staphyllococcus spp.* which produced enzyme called beta-lactamase, acting on the part of structure of penicillin, causing the lost of antimicrobial activity (Meyer *et al.*, 1980). Drug resistance is the reason for medicinal development. The newer drugs were discovered by changing chemical structure or molecular structure (Grayson, 1982). Methicillin, cloxacillin, flucloxacillin were synthesized to cover gram positive resistance strains, but not the gram negative bacteria. Then ampicillin, epicillin and hetacillin were introduced for broader spectrum against gram negative and gram positive bacteria (Grayson, 1982).

There are many infections with underlying severity of illness such as pneumonia, leukemia, septicemia and intraabdominal sepsis (Lutz and Mogabgab, 1978, Hewitt *et al.*, 1978). The most striking finding is that the majority of these infections were caused by gram negative rods espiacially *Pseudomonas aeruginosa* and *Enterobacteriaceae*. Infections continues to be a major cause of morbidity and mortality among hospitalized patients. No antibiotics have been entirely satisfied for treatment of these infections. The aminoglycosides provides a broad spectrum activity with nephrotoxicity and appeared to to be somewhat less effective in compromized host. The penicillins are less toxic and are the principal choice for these infections. Carbenicillin is the first member of this drug group. (Bodey and Le

Blanc., 1978), its activity against *Pseudomonas aeruginosa* is poor in comparison with its activity against *Enterobacteriaceae*. Ticarcillin may be considered to be the modest advance over carbenicillin. Azlocillin and mezlocillin are the two-α-amino substitued penicillins with broad spectrum and antipseudomonal activity. Piperacillin is another member of the structurally related group of penicillin (Wise *et al.*, 1981; Bodey and Le Blanc ., 1978). It has a broader spectrum than the others against all of the gram negative bacteria (White *et al.*, 1979; Baier and Puppel., 1979; Shah *et al.*, 1979).

Chemistry

Piperacillin is sodium-6-[D-(-)α-4 Ethyl-2,3-dioxo-1 piperazinyl carbonylamine-α-phenylacetamido penicillinate derived from 6-amino penicillanic acid nucleus (Figure 1).

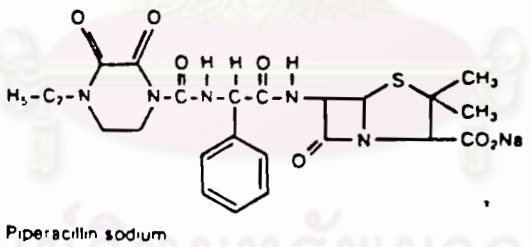

Fig. 1 Basic chemical structure of piperacillin (Fu and Neu, 1978)

Piperacillin sodium 1.04 gm is approximately equivalent to 1 gm of piperacillin. It is soluble in 1 to 1.14 of water and methyl alcohol and 1 in 5 of alcohol. Its melting point is 180°C. Piperacillin sodium powder will maintain at least 90% of labelled potency (L.P.) when stored at controlled room temperature (23°C) for 3 years. The drug is stable in solution at pH range 4.5 to 8.5. Reconstitued

piperacillin sodium has the proven stability over the concentration range 0.2% to 40% in various container, both glass ans plastic under various storage conditious. Vial of piperacillin sodium was reconstituted with sterile water for injection, bacteriostatic water for injection, or bacteriostatic sodium chloride injeciton. Each of the three solutions maintained at least 90% of labelled potency (L.P.) when stored for 24 hours at controlled room temperature, 1 week under refrigerated conditions and 1 month when frozen. The incompatibility is none among standard intravenous diluents (Reynold, 1982).

Antibacterial activity

Piperacillin like other penicillins and cephalosporins are susceptible to the action of some beta-lactamase. It has a broad spectrum of activity in vitro which included gram positive bacteria (*Enterococci*, non-beta-lactamase produding *Staphyllococcus aureus* and *Staphyllococcus* epidermidis, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and other beta hemolytic *Streptococcus*), gram negative bacteria (*Acinetobacter spp.*, *Neisseria gonorrhoeae* included beta lactamase, *Neisseria menigitidis* and *Enterobacteriaceae*) and anaerobic bacteria (*Bacteroides fragilis*, *Bacteroides spp.*, *Clostridium difficile*, *Clostridium spp.*, *Eubacterium spp.*, *Fusobacterium spp.*, *Peptococcus spp.*, *Peptostreptococcus spp.*, *Veillonella spp.*)(Fu and Neu, 1978; Verbist *et al.*, 1978, Bodey and Le Blanc, 1978, Lederlle on file).

All gram positive cocci excepted penicillin resistant *S. aureus* were inhibited by 0.78 µg/ml of piperacillin (Bodey and Le Blanc, 1978). 0.1-0.12 µg/ml Piperacillin inhibited *Streptococci* but

not the *Enterococci*. Non β-lactamase producing *Staphyllococci* and *Enterococci* were also inhibited by 2 µg/ml piperacillin (Fu and Neu, 1978; Verbist, 1978). Its activity against *Staphyllococcus aureus* is equivalent to mezlocillin and carbenicillin but slightly less than ampicillin (Fu and Neu, 1978). *Streptococci*, particularly *Streptococcus pneumoniae*, show similar high sensitivity rates to piperacillin as to other newer penicillins. None of the newer penicillins had an advantage over ampicillin for *Enterococci* (White *et al.*, 1979).

Piperacillin exhibited a wider spectrum of activity and in some cases greater potency against gram-negative micro organisms than other member of penicillin group (Dickinson *et al.*, 1978; Baier and Puppel, 1980; Shah *et al.*, 1979; Mc.Gowan *et al.*, 1979; Barry *et al.*, 1979). At concentration of 25 µg/ml, Piperacillin inhibited 83% of *Citrobacter spp.*, 58% of *Klebsiella spp.*, 85% of *Enterobacter spp.*, and 50% of *Indole positive proteus*, *Acinetobacter* and *Providencia* (Fu and Neu, 1978). Most *Proteus spp* were extremely susceptible to piperacillin, over 85% were inhibited by 0.10 µg/ml (Bodey and Le Blanc, 1978). As with other penicillins, piperacillin possed little activity against *Klebsiella pneumoniae* strains (Shah *et al.*, 1979). *Klebsiella spp* showed a higher sensitivity to cefoxitin and cefuroxime (Baier and Pupple, 1980). *Haemophilus influenzae* was highly susceptible to all of the penicillins (ampicillin, ticarcillin, azlocillin, mezlocillin)(White *et al.*, 1979). *Pseudomonas aeruginosa* has shwon greater susceptibility to piperacillin than other penicillin. Antipseudomonas activity of this penicillin could be demonstrated as follows : piperacillin > azlocillin > mezlocillin = ticarcillin > carbenicillin (White *et al.*, 1979; Wise *et al.*, 1978; Shah *et al.*,

1979; Mc Gowan *et al.*, 1979; Bodey and Le Blanc, 1978).

At concentration of 8 µg/ml or less piperacillin inhibited 90% of most strains of anaerobic bacteria, between 1 and 2 µg/ml inhibited 50% of Bacteroid fragilis (Wise *et al.*, 1978) and 25 µg/ml inhibited 78% of this species (Fu and Neu, 1978).

The minimum inhibitory concentration of piperacillin against *Ps. aeruginosa* was affected by increasing the inoculum size (Fu and Neu, 1978). Large increase in MBC with large inocula (10^7 CFU/ml) showed the inoculum effect on MIC and MBC in five isolation of five difference micro-organism (*E. coli*, *Klebsiella spp.*, *P. mirabilis*, *S. marcescens* and *Ps. aeruginosa*)(Verbist *et al.*, 1978).

Synergistic activity of piperacillin was demonstrated when combined with amikacin, gentamicin and azlocillin against *Ps. aeruginosa* and member of *Enterobacteriaceae* (Fu and Neu, 1978; White *et al.*, 1979). Combination of piperacillin and tobramicin was the most active penicillin-aminoglycoside combinations tested for synergism (Shah *et al.*, 1979). Synergistic activity of piperacillin in combination with β-lactamase inhibitors (Clavulenic a and penicillinic acid sulfone) inhibited various *Enterobacteriaceae*, *Staphylococcus aureus* and *Bacteroid fragilis* (Neu and Fu, 1980). Cefoxitin combined with piperacillin resulted in an increase in the MIC of piperacillin (Busch *et al.*, 1980; Kuch *et al.*, 1981; Sander *et al.*, 1982).

Although piperacillin has some stability to chromosomally mediated β-lactamases (Leigh and Simon, 1979), it is similar to mezlocillin in that it is hydrolysed by the plasmid-mediated β-lactanase,

in particular the TEM-1 enzyme (Fu and Neu, 1978, Wise *et al.*, 1981). The parallel resistance exist between piperacillin and other antibiotics such as ampicillin and carbenicillin. For Carbenicillin, in isolates *Pseudomonas aeruginosa* the correlation coefficient of linear regression was 0.923 (Verbist, 1978).

Pharmacokinetics

1) Absorbtion and serum concentration

Immediately after an intravenous bolus of 4 g piperacillin mean peak plasma concentrations in healthy subjects were within the range of 330 to 412 µg/ml (De Schepper *et al.*, 1982; Tjandra Maga, 1980). The same dose given by infusion over 30 minetes to healthy subjects resulted in a mean peak concentration of 244 µg/ml (Batra *et al.*, 1979). Slow bolus injection over 3 minutes of 1,2,4 and 6 g produced average peak concentrations from about 70 to 500 µg/ml. Disproportion of low serum levels were found after the low intravenous doses. Upon doubling the dose from 1 to 2 g, area under the plasma concentration time curve (AUC) increased nearly 3 folds (from 36 to 102) while increasing the dose from 1 to 6 g may associate with 12 folds increase in AUC (from 36 to 437 µg/ml). Intramuscular injection of 0.5, 1 and 2 g resulted in 70 to 80% bioavailability of piperacillin and mean peak serum concentration range from 30 to 40 µg/ml after 2 g dose in heathy subjects and patients. Average peak plasma concentration reached within 45 minutes after 0.5, 1 and 2 g intramuscular injection were 5, 13 and 30 µg/ml, respectively (Tjandra Maga, 1980).

2) Distribution

The average apparent volume of distribution at steady state (Vd) of piperacillin was 19 L/1.73 m^2 after 1 g dose and 16 L/ 1.73 m^2 after 6 g intravenous bolus dose. Distribution phase half life after 1-6 g intravenous doses of piperacillin ranged from 0.17 to 0.32 hours (Tjandra-Maga *et al.*, 1978).

The concentration of piperacillin in cerebrospinal fluid (CSF) of infants and adults with meningitis approached one-third of the level maintained in the serum by continuous infusion (Dickinson *et al.*, 1981; Rubio *et al.*, 1982; Hoogkamp-Korstanje, 1982; Shishido and Matsumoto, 1979; Decazes *et al.*, 1984).

A mean concentration of piperacillin 31.4 µg/ml was achieved in bronchial secretions 30 to 45 minutes after intravenous administration of a 4 g dose and the sputum/serum ratio was approximately 15%. Penetration into pleural fluid in 1 patient rose from 9 to 26% after 4 and 28 doses of 2 g piperacillin, whilst a second patient achieve 47% penetration after 8 doses of piperacillin 2 g (Marlin *et al.*, 1981).

Concentration of piperacillin was likely to be effective against most organisms in the uterus, ovary, oviduct, myometrium, endometrium, portovaginalis and cervix uteri 0.5 to 4 h after intravenous administration of 1 to 4 g piperacillin (Kusuhara *et al.*, 1982; Nakamura *et al.*, 1982; Weissenbacher *et al.*, 1982). Penetration into amniotic fluid has not been reported to be as high as that into umbilical cord 71% vs 24% approximately 1 hour after an intravenous dose of 1 g piperacillin (Twasaki and Machihara, 1982).

About 20% of piperacillin dose was excreted through the biliary tract, producing biliary concentrations up to 40 times compare to that in serum (Russo *et al.*, 1982).

3) Protein binding

The extent of protein binding of piperacillin was around 21% (Batra *et al.*, 1979) which was similar to the values reported for mezlocillin, azlocillin and ampicillin but lower than that of other β-lactamase antibiotics.

4) Elimination

In patients with normal renal function, piperacillin is eliminated primarily (80%) by glomerular filtration and tubular secretion. High urinary level, 60 to 80% of intravenously administered doses were recorded unchanged in the urine. These values were higher than those obtained after intramuscular administration (57% after 1 g dose)(Tjandra-Maga *et al.*, 1978).

The average half life of piperacillin in healthy volunteers was dose dependent from 0.6 to 1.05 hours after intravenous administration of 1 and 6 g respectively. Similarly, following intramuscular administration, terminal half life were 1 hour after 0.5 g, 1.15 hours after 1 g and 1.34 hours after 2 g doses (Tjandra-Maga *et al.*, 1978).

Clinical Trial

In clinical studies of piperacillin is known to be effective in the treatment of aerobic and anaerobic infections. Piperacillin was shown clinically to be safe and effective antibiotic to treat

serious infection due to gram negative bacteria. It appeared to be very active against *Ps. aeruginosa*, many of which were carbenicillin resistance.

Piperacillin given 1 to 18 g daily, was effective in the treatment of urinary-tract infection. Overall clinical response rates for piperacillin in bacteriological response were 51 to 83% (Clark 1980, Gooding *et al.*, 1982).

Two of the three comparative trials of piperacillin and carbenicillin, found that these two antibiotics were similar in clinical and bacteriological efficacy (Alftan and Renkonen, 1982; Marier *et al.*, 1982). The remaining study, however, shown the significant superiority of piperacillin against various urinary tract infections (Kawada *et al.*, 1977).

The percentage eradication of bacterial strains isolated from patients having complicated urinary tract infections by piperacillin varied widely between different gram positive and gram negative organisms (Tunn, 1980; Hasekawa and Kanda, 1977).

In patients with respiratory tract infections, including pneumonia, a clinical response rate was about 90% in opened studies of piperacillin (2 to 16 g, daily)(Kato *et al.*, 1977; Machette, 1981; Pancoast *et al.*, 1981). In addition to being clinically effective against *S. pneumoniae* and *H. influenzae*, piperacillin was also effective in a small number of patients with respiratory tract infections caused by other Gram-negative bacteria (*K. pneumoniae*, *E. coli*, *P. aeruginosa*, *Proteus mirabilis*). Piperacillin was more

effective than ampicillin in cases of bacterial pneumonia and chronic respiratory tract infections (Nakagawa *et al.*, 1978). The overall clinical efficacy rate was also significantly greater in the piperacillin group for all the different cases of treated respiratory tract infection. Piperacillin has been used with intermediate success in *P. aeruginosa* infections in patients with cystic fibrosis (Agostini *et al.*, 1983).

Opened studies sucessful treatment with piperacillin in various gynaecological and obstetric infections was about 90% of the patients (Privitera *et al.*, 1983; Broer, 1980; Cho *et al.*, 1977). The Initial doses of 2 to 8 g daily were used but now a slightly higher dose is recommened within the range 4 to 12 g daily. Piperacillin (4.5 g - 6 g/6 hours) was found to be as effective as cefoxitin (2 g intravenously/6 hours) in patients with upper genital tract infection (Sweet *et al.*, 1982).

Patients with bacteremia have responded to piperacillin therapy (up to 12 g daily) with clinical cure/improvement rates of 85 to 95% (Humphreys, 1980; Clark, 1980; Gooding *et al.*, 1982). However piperacillin 24 g/day in combination with tobramycin 8 mg/kg/day failed to cure infective endocarditis due to *Pseudomonas aeruginosa* in 6 of the 8 treated cases (Reyer and Lerner, 1981).

Single intramuscular injecitons of 2 g piperacillin yielded the cure rate of 95 to 100% (Lancaster *et al.*, 1981; Simpson *et al.*, 1982). It was as effective as procaine penicillin G. 4.8 million units in cases of uncomplicated gonorrhoea cause by non-penicillinase producing organisms (Landis *et al.*, 1981). Cure rates in patients

patients with *penicillinase producing gonorrhoeae* ranged from 78 to 96% (Lancaster *et al.*, 1981).

Treatment of intra-abdominal sepsis with piperacillin has resulted in a satisfactory clinical response in 70 to 90% of cases, (Clark, 1980; Fredlund *et al.*, 1982; Gooding *et al.*, 1982). None of the patients were treated prophylactically with piperacillin for 3 to 8 day in an uncontrolled study of developed post operative infection (Jikuya *et al.*, 1977). Piperacillin (18 g daily for 7 days) was found to be as effective as cefoxitin (8 g daily for 8 days) in the treatment of surgical abdominal infections (Najem *et al.*, 1983).

Immunocompromised patients responded well to various combinations of piperacillin with moxalactam, amikacin, netilmicin or cefmetazole as other combinations of ticarcillin with cephalosporins aminoglycosides (Wade *et al.*, 1980; Winston *et al.*, 1982). Overall response rates averaged 74% for the combinations of piperacillin with another antibiotic (de Jongh *et al.*, 1982).

Bone and joint infecitons have responded to piperacillin therapy with clinical cure rate of 84 to 87% (Clark, 1980), and 90-95% for skin and soft tissue infections (Clark, 1980; Gooding *et al.*, 1982; Humphreys, 1980)

A few studies of piperacillin alone or in combination with an aminoglycoside have produced satisfactory responses in a small number of neonates, children, and adults with gram negative bacillary meningitis (Hoogkamp-korstanji, 1982).

Side Effect

Piperacillin is generally well tolerated, the most commonly reported side effects are similar to those of other penicillins including local reactions, gastrointestinal, haematological, hepatic or renal effects (Stead *et al.*, 1984; Møller and Høiby, 1981; Gooding *et al.*, 1982; Clark, 1980; Humphrey 1980). Occasional findings of the alteration in platelet function (Gentry *et al.*, 1981) and hypokalemia have also been reported (Wade *et al.*, 1980).

Dosage and Administration (Manufacturer recommendation)

Piperacillin can be administered intravenously or intramusculary. For adults with less serious and uncomplicated infections the total daily dose range between 6 and 8 g/day. More complicated and serious infection require a higher dosage, ranging between 12 and 18 g/day with a maximum around 24 g/day. Less serious infections in infants can be treated with 100 to 200 mg/kg/day while severe infection may require 100 to 300 mg/kg/day.

Aim of the thesis

In selecting antimicrobial agent for effective therapy in critically ill patients, these drugs must share essential characteristics namely in vivo as well as in vitro effectiveness and lack of toxicity (Moellering, 1981). According to the reports of new drug from manufacturer or foreign countries mostly revealed the good results. When using these recommendations for treatment of serious infection in Thai patients, the drugs did not show effectiveness as reported

the references. Sometime the clinician was bewildered by multiplicity of names and the strident claims in the promotional literature.

Piperacillin (T. 1220) was also the new drug which recently (1984) introduced by Lederlle Co. Ltd. (Thailand) for the treatment of serious infections in Thai patients. Piperacillin was semi-synthetic penicillin that had favorable in vitro and in vivo effectiveness with non-serious side effects excepted common adverse reactions to penicillins. At the time of this study begun (1984), piperacillin was only in vitro and clinical trials, although it was the post marketing surveillance for manufacturer company.

As there has not been any reports about piperacillin in our country yet, therefore the main aims of this study were to evaluate the in vitro antibacterial activity of piperacillin against gram negative bacteria and its therapeutic efficacy in Thai paedriatic patients with severe infections. These aims were achieved by doing the following studies :-

1) Study of the in vitro antibacterial activity of piperacillin against gram-negative bacteria which were clinically isolated from three hospital centers in Bangkok (Ramathibodi, Chula longkorn, Rajvithi) and *Pseudomonas pseudomallii* from Ubolrajthani hospital.

2) Study of the clinical and bacteriological efficacy of piperacillin against severe bacterial infections in paedriatic patients at Ramathibodi hospital.

3) Study of the adverse effect of piperacillin

4) Study of the pharmacokinetics after the intravenous administiation of piperacillin. Serum drug level at time 10 min, $\frac{1}{2}$ - 1, 2-4 and 6 hours after intravenous administration was also studied in patients.