CHAPTER V

CONCLUSIONS

The results were concluded as follows;

1. Development of HPLC method

A simple and selective high performanced liquid chromatographic method for the determination of cefoperazone and sulbactam in human plasma samples has been developed and validated. Cefoperazone and rosiglitazone (internal standard) were extracted from human plasma by liquid-liquid extraction using methanol. The analysed were performed on a OSD Hypersil® C18, 250x4 mm, 5µ column, using a mixture of acetonitrile: methanol: 5mM tetrabutylammoniam hydroxide (13:9:78), pH 6.8 as a mobile phase with UV detection at 220 nm. For determination of sulbactam and enalapril (internal standard), were extracted by liquid-liquid extraction using diethyl ether. The analysed were performed on a OSD Hypersil® C18, 250x4 column, using a mixture of acetonitrile: 5mM tetrabutylammoniam hydroxide (25:75), pH 6.5 as a mobile phase with UV detection at 220 nm. The validated methods were successfully applied for bioequivalence studies in human.

2. Comparison pharmacokinetic parameters of recommended dosage regimen of cefoperazone/sulbactam in HAP patients

Thirty-two hospital-acquired pneumonia patients completed the study. The three recommended cefoperazone/sulbactam regimens were comparable. Eight patients (25.0%) were given cefoperazone/sulbactam (500mg/500 mg) 1g I.V. q 12 h. 18 patients (56.3%) and 6 patients (18.8%) were given cefoperazone/sulbactam 2 g I.V. q 12 h and 2g I.V. q 8 h, respectively. The range age was 17-82 years old (59.38±15.91). Most of the patients were elderly with serious illness (62.1% were ICU or sub ICU patients) and showed evidence of renal and hepatic compromise. For cefoperazone, mean AUC_{0-t} of cefoperazone/sulbactam 1 g q 12 h, 2 g q q2h and 2 g q 8h were 324.95, 742.5 and 568.77 µg.h/ml, respectively. The Vd were 12.31, 15.91, and 20.67 L. The half lives of cefoperazone were 5.19, 7.45, and 7.22 h, respectively. From analysis of variance of the pharmacokinetic parameter, Vd, Ke, T1/2 ,and CL presented there were no significant differences in each dosage regimens, except the AUC_{0-t}, showed the significant difference between dosage regimen.

For sulbactam, mean AUC0-t of cefoperazone/sulbactam 1 g q 12 h, 2 g q q2h and 2 g q 8h were 96.02, 241.83, and 170.99 µg.h/ml, respectively. The Vd were 21.45, 25.02 , and 22.39 L, respectively. The half lives of cefoperazone were 1.22 , 3.64, and 2.32 h, respectively. From analysis of variance of the pharmacokinetic parameter, Vd, Ke, T1/2 ,and

CL presented there were no significant differences in each dosage regimens, except the AUC_{0-t}, which were significant difference between dosage regimen.

2. MIC distribution of *A.buamannii* and *P.aeruginosa*.

All 149 isolate of *A.baumannii*, and 215 isolates of *P.aeruginosa* were obtained from true sputum or tracheal secretion of patients in Maharaj Nakorn Chiang Mai hospital. MIC data for each isolates were determined by the E-test method.

For *A.buamanii*, 58.3% of isolates was susceptible to cefoperazone/sulbactam and 29.2%% was intermediate and 12.5% was resistance. MIC frequency values for *A.baumannii* of cefoperazone/sulbactam ranged from 1 to 256 μg/ml. The percentage of isolates at MIC < 16 μg/ml were 54.2 % (susceptible), 16-48 μg/ml were 31.40 % (intermediate) and >64 μg/ml were 14.4 % (resistant). *P.aeruginosa* was susceptible to cefoperazone/sulbactam 53.0% of isolates. MIC frequency values for *P.aeruginosa* ranged from 1.0 to 256 μg/ml for *P.aeruginosa*. The percentage of isolates at MIC <16 μg/ml were 60.4% (susceptible), 16-48 μg/ml were 21.5 % (intermediate) and >64 μg/ml were 18.1 % (resistant).

3. Monte Carlo simulation for cefoperazone/sulbactam

A 5,000-patient Monte Carlo simulation was simulated to calculated estimates of %T>MIC and %*f*T>MIC for each cefoperazone/sulbactam regimen against *A.baumannii* and *P.aeruginosa*. Bactericidal pharmacodynamic targets of cefoperazone/sulbactam were defined as 50% *f*T>MIC. A regimen that achieved >90% CFR against a population of organism was considered optimal for empirical treatment.

According to 50% total time above MIC as a bactericidal pharmacodynamic target, we found that cefoperazone/sulbactam 2 g q 12 h and 2 g q 8 h provided a highest CFR for both *A.baumannii* and *P.aeruginosa*. (72.76% and 72.84% ,respectively for A.baumannii, For P.aeruginosa, 76.8 and 77.88%, respectively), meanwhile cefoperazone 1 g q 12 h displayed a lower CFR (52.58 and 63.0% for *A.buamannii* and *P.aeruginosa*, respectively.)

From the simulation, the highest MIC for which target is attained by at least 90% of the simulated subjected is defined PK-PD breakpoint. Both of the regimen 2 g q 12 h and 2 g q 8 h achieved a greater than 90% probability at MIC 24 μg/ml which were better than regimen 1 g q 12 h which achieved at 10 μg/ml. Thus regimen 2 g q 12 h and 2 g q 8 h can be achieved the 90% at MIC < 24 μg/ml

Although the regimen 2 g q 12 h and 2 g q 8 h of cefopeazone/sulbactam had a high bactericidal CFR, there could not be achieved target at 90% CFR. Thus, in this study, the alternative dosage regimens were simulated to study in dosage regimen of 1000 mg of cefoperazone q 6 h and 2000 mg of cefoperazone q 12 and 8 h. The simulation showed the bactericidal CFR increased to 80.44, 88.88, and 89.69%, respectively for *A.buamannii* and 76.88, 82.78, and 83.16% for *P.aeruginosa*. However there were no regimens achieved at 90% of CFR. Therefore, the recommended cefoperazone/sulbactam 2 g q 12 h or 2 g q 8 h should be used for empirical treatment HAP in this hospital because there had a high bactericidal CFR and there were no significant different between the recommended dosing regimens and the alternative dosing regimen. However, the use of combination therapy with cefoperazone/ sulbactam should be justified.

Many previous studies, reported the percent of free concentration exceeds MIC is more closely to the clinical outcome. Thus, this study we also simulated the regimens to determined the free drug concentration above the MIC. There were no recommended regimen was able to attain 90% CFR against this population of *A.baumannii* and *P.aeruginosa*. The greatest CFRs were attained by cefoperazone/sulbactam (500mg/500mg) 2 g I.V. q 8 h, were 20.72% CFR for *A.baumannii* and 26.1% CFR for *P.aeruginosa*. As a result, there were very low CFR achieved the bactericidal target in any recommended dosage regimens. The simulations in this study demonstrated that based on free drug concentration above MIC, the recommended cefoperazone/sulbactam (500mg/500mg) dosage regimens should not be an empirical drug treatment for *A.baumannii* and *P.aeruginosa* infection in this hospital.

Alternative dosage regimens were simulated from pharmacokinetic parameters in dosage regimens of 1000 mg of cefoperazone every 6 h and 1000 mg of cefoperazone every 8 h. When the cefoperazone/sulbactam was increased to 1000 mg of cefoperazone q 6 h and 2000 mg of cefoperazone injection every 12 h and every 8 h, the bactericidal CFR slightly increased to 22.3% ,27.98 and 29.14 %respectively for *A.baumannii* organism, and increased to 26.6 , 45.7 and 46.5%, respectively for *P.aeruginosa* organism. Although the doses were increased, the bactericidal CFR were less than 50% CFR. Thus based on free T>MIC, there were no regimen can be an empirical treatment in this hospital.

There are many factor that can altered the free plasma levels in hospital-acquired pneumonia patient, especially in cefoperazone which had a highly protein binding 90%. Unfortunately, we were not determined the free concentration of cefoperazone, so in the equation which allowed to use free fraction unbound 0.1 of cefoperazone may be not related to the free concentration in these patients.

4. Identify factors associated with clinical outcome of treatment hospital-acquired pneumonia with cefoperazone-sulbactam

Twenty eight patients were assessed for a clinical and microbiological response. All evaluations were performed on day 1, day 3, day 7 or the end of cefoperazone-sulbactam treatment. At the end of treatment, clinical cure was note in 25 % of patients (7/28), improvement 42.9% (12/28) and 32.1% (9/28) had clinical failure. For microbiology outcome, microbilogical eradication was note in 13 /28 (46.4%), 11/28 patients had organism persistence and 4 patients had new infection organism.

In chi-square analyse, we found that cefoperazone/sulbactam total cefoperazone concentration exceed the MIC (50% T>MIC) and age less than 60 years were significantly associated with clinical response (p=0.041, (OR=6.8) and p=0.01, (OR=13.71),respectively). Meanwhile, the 50% free cefoperazone concentration above MIC was not significant associated with the clinical response.