CHAPTER III

MATERIALS AND METHOD

The study was divided into 2 parts

- Retrospective Study
- Prospective Study

Retrospective Study

1. Materials

1.1 Medical records of patients, treated with CAP, admitted to the Department of Medicine, Pediatrics, Surgery and Gynecology, Ramathibodi Hospital, Bangkok, from January 1, to December 31, 1980 were included in the study.

1.2 Medication and adverse drug reaction recording data

2. Method

2.1 reviewed medical record of every patient treated with CAP.

2.2 Divided all patient records into several groups according to following conditions;

2.2.1 Duration of CAP treatment. They were divided into 5 groups:

a. 1 - 7 days

b. 8 - 14 days

c. 15-21 days

d. 22-35 days

e. more than 35 days

2.2.2 Age of patients, they were

a. 1-15 years, this age group was subdivided into 5 subgroups as described below:

i less than 1 year

ii 1-2 years

iii 2-5 years

iv 5-10 years

v 10-15 years

b. 15-30 years

c. 30-45 years

d. 45-60 years

e. more than 60 years

2.2.3 Dosage of CAP therapy, they were

a. 1 g/day (or 25 mg/kg/day)

b. 2 g/day (or 25-50 mg/kg/day)

c. 3 g/day (or 50-75 mg/kg/day)

d. 4 g/day (or 75-100 mg/kg/day)

2.3 Recorded all suspected adverse effects on the medication and adverse drug reaction recording data. All reactions were classified as:

Probably related; if there was no other cause for the reaction apparent.

Possibly related ; if there was another cause which may have been responsible

Doubtfully related ; if there was another cause which was more likely responsible.

2.4 Analyzed collected data by statistic method, if possible

Prospective Study

1. Materials

1.1 Study Patients

Serum levels and ADR studies following the administration of CAP were performed in 33 pediatric patients, aged from 5 months to 13 years, treated at pediatric ward I, II and IV, Ramathibodi Hospital from October, 1981 to March, 1982.

1.2 Patient hospital charts

1.3 Medication and adverse drug reaction recording data

1.4 Medication

CAP were obtained in 3 different dosage forms of

a. CAP succinate esters (CAP-S) were manufactured by Carlo Erba,Italy. Each vial contains 1 g of CAP-S, it must be dissolved in 5 ml of sterile water for injection prior to use.

b. CAP capsules were manufactured by Lepetit Laboratories, Thailand.Each capsule contains 250 mg of CAP base.

c. CAP glycinate esters (CAP-G), batch no 15060; manufactured by Zambon Laboratories,Italy, was obtained from distributor in Thailand. Each vial contains 1 g of CAP-G, it must be dissolved in 5 ml of sterile water for injection prior to use.

1.5 Apparatus and reagents for microbioassay.

a. Culture medium.

Trypticase soy agar (Difco Laboratories, Detriot Michigan,U.S.A) with 4% sodium chloride (May and Baker Laboratories Chemicals,Dagenham, England).

b. Diluent

Phosphate buffer saline (PBS) pH 7.2

c. Test organism

Sarcina lutea ATCC 9341

d. CAP curve

Five standard concentrations; 5,10,20,40,80 mcg/ml were obtained and 10 mcg/ml was the reference point.

e. Standard CAP powder

CAP base, batch number 1489, with expired date on 15 April 83, was obtained from Parke-Davis Laboratories(Thailand).

f. Penicillinase solution (Difco Laboratories,Detroit Michigan, U.S.A). Each ml contained 2,000 I.U. of penicillinase and 8 mcl of this solution should be used for incubating 1 ml of specimem.

g. Samples.

Two ml of blood, one ml of CSF and suitable volume of urine were obtained for CAP level assay.

h. Equipment for assay.

- Finnpipette (manufactuered by Finnpipette Helsinki)
- Cork borrer
- Vernier caliper
- Pyrex petridishes size 100 X 15 mm.

2. Method

2.1 Detailed design for study

A. Dosage guildline for all patients.

age	Dosage
6-13 years	50-70 mg/kg/day every 6 hours
2-6 years	50-100mg/kg/day every 6 hours
∠ 2 years	100mg/kg/day every 6 hours

B. Route of administration

CAP-S was administered by intravenous infusion while CAP-G was administered by either intramuscular injection or intravenous infusion. CAP base was administered orally. other drug might ocassionally be administered simultaneously into the same injection or infusion site.

C. Collection of specimens.

Blood samples were collected, when possible, before and at 30 to 45 minutes after first intravenous and every 7-10 days while continuing with the same route of administration. If the treatment was changed to another route of CAP administration, such as; IM and/or oral administration, blood sample had to be collected again thereafter at the timing which was supposed to be peak serum level of that route of administration. Therefore, blood sample had to be collected before and at 45-60 minutes after IM, and thus before and 2 hours after oral administration. Blood samples were allowed to clot and were centrifuged, then the

serum were promptly removed for assay.

CSF samples were collected at 45-60 minutes after IV administration, if possible.

Urine samples were collected at 1 and 2 hours after IV and oral administration respectively. The samples had to be diluted to 1:40 and 1:80 dilution, with PBS, before assay.

Serum, CSF and urine samples were stored frozen (-20°C) until assayed. CAP concentration were measured by the agar dilution method.

D. Duration of treatment

Duration of CAP therapy was based on the patients clinical course. Therapy was stopped if the infection failed to response to CAP, or if a toxic or allergic reaction occurred.

2.2 Microbioassay for CAP concentration

A. Preparation of standard concentration

Potency of reference standard powder was 0.994 mg per 1 mg powder. So 1000 mcg/ml of stock standard solution was prepared by weighing out 0.1010 g of stock known potency powder. This was placed in a 100 ml of volumetric flask and 2 to 3 ml of ethanol was addeded for dissolving the material. The flask was then made up to 100 ml with PBS pH 7.2. This stock solution could be kept deep frozen for several weeks.

From the stock solution five standard solution were prepared by following procedure;

i. 0.4 ml of stock solution + 4.6 ml of PBS = 80 mcg/ml

ii. 2 ml of (i) + 2 ml of PBS = 40 mcg/ml

iii. 2 ml of (i) + 2 ml of PBS = 20 mcg/ml

iv. 2 ml of (i) + 2 ml of PBS = 10 mcg/ml

v. 1 ml of (i) + 1 ml of PBS = 5 mcg/ml

B. Preparation of test organism

Sarcina lutea ATCC 9341 was grown in nutrient agar slant overnight. Then a suspension of Sarcina lutea was prepared by using sterile glass bead rolling, with PBS, in S.lutea agar slant tube, then the suspension was poured out and adjusted to an transmittance of 32-34 at 580 nm by using PBS as diluent.

C. Preparation of assay agar

The assay agar was prepared by mixture of 40 g of trypticase soy agar and 40 g of sodium chloride with one litre of distilled water. The mixture was brought to the boiling point until the media cleared and was then autoclaved

D. Procedure of CAP assay [67]

-0.5 ml of Sarcina lutea suspension was added to 100 ml of melted agar kept at 50°C

-The seed agar (12 ml) is immidiately poured into 100 X 15 mm standard pyrex petridishes and allowed to solidify on a level surface.

-Each specimen was incubated at 35°C 15 mimutes with penicillinase solution (16 I.U. per 1 ml of specimen).

-The assay, a modification of agar-paper disk diffusion method of Sabath et al[69] was performed by pipetting of 50 mcl of each penicillinase treated specimen into a well of a diameter of 9 mm, which was in theseeded agar plate.

-Each sample was treated in triplicate and each standard solution was treated in duplicate in each plate

-The diameter of the zone of growth inhibition was measured, by vernier caliper, after incubation for 20 hours at 35°C

-Results were calculated by plotting the zone diameter against the antibiotic concentration on semi-logarithmic paper where a curve approximately to a straight line should be obtained (71)

E. Concomitant medication

CAP was often prescribed in combination with other drugs such as penicillin, sulphonamide or aminoglycosides (eg.gentamicin, kanamycin, amikacin). The first two of these drugs can be inactivated by a good penicillinase preparation or p-aminobenzoic acid respectively.(70) But in this study the combination of CAP and sulphonamide therapy were excluded because a good p-aminobenzoic acid preparation was not available

The presence of aminoglycosides, would interfere the inhibition zone of CAP, was eliminated by adding 4% sodium chloride in assay agar(69) as already mentioned in preparation of assay agar.

2.3 Monitoring for adverse effect

A. Patient status

Recorded the information of each patient. Most of the information was obtained from patients hospital charts after the ward physician had completed his examination. Patients admitted to these pediatric wards,were cared for by interns, residents, and physician staff as chief wards. The specialist were the consultants of the wards.

B. Laboratory test

The following laboratory test of hematopoietic, hepatic and renal function were done :

Hemoglobin level

Hematocrit value

Total leukocyte count

Differential leukocyte count

Serum glutamic-oxaloacetic transaminase level (SGOT)

Alkaline phosphatase level

Serum creatinine concentration

Blood urea nitrogen level (BUN)

Complete urinalysis with microscopic examination of the sediments

Special test for specific adverse effects were done as appropriate, for example, reticulocyte count, prothrombin time, thrombin time, partial thromboplastin time and platelet count.

Laboratory test listed above, especially complete blood count, were performed every 7-10 day during CAP therapy, in patients who had received CAP more than 14 days.

C. Criteria for evaluation of changes in hematopoietic function

If white blood cell (WBC) was less than 5,000 cell/mm^3 and/or neutrophil was lower than 40%, the adverse effect must be suspected

2.4 Analysis of data

A. Comparison of CAP-S and CAP-G

Comparison of serum CAP level after CAP-S and CAP-G

administration was performed in children aged from 2-13 years. Differences in mean were analyzed by t-test.

B. Evaluation for adverse effects.

The incidence of adverse effect on bone marrow cell, gray syndrome and those reactions which might be considered to be related to CAP, might be further analyzed if appropriate. Sex, age, serum level and duration of treatment had to be considered in this analysis by chi-square test and ANOVA.

C. Evaluation of CAP concentration in CSF and urine

Differences in mean were analyzed by t-test, if possible.