CHAPTER III

MATERIALS AND METHOD

METERIALS

1. TDx[R] Gentamicin

1.1 No. 9512-01, Gentamicin Calibrators

Six vials with accurately measured amounts of gentamicin in normal human serum were prepared as follows

Vial	Gentamicin Concentration(µg/ml)
A	0
B	0.5
C	1.5
D	3.0
E	6.0
F	10.0

1.2 No 9512, Gentamicin controls

Three vials of gentamicin in normal human serum should give the reading within the following concentration ranges.

Vial	Gentamicin concentration(μg/ml)	
L	0.85-1.15	for 1
M	3.6-4.4	for 4
H	7.2-8.8	for 5

(Preservative : 0.1% sodium azide)

1.3 No. 9512-20, Gentamicin Reagent Pack

There are three vials of gentamicin reagent pack

Vial

P Pretreatment Solution : Surfactant in buffer containing protein stabilizer(3 ml)
Preservative : 0.1% Sodium azide

S Gentamicin antiserum (sheep) in buffer with protein stabilizer
Preservative : 0.1% sodium azide

T Gentamicin-fluorescein tracer in buffer containing surfactant and protein stabilizer (3 ml)
Preservative : 0.1% Sodium azide

1.4 No. 9512-20, Buffer
(for in vitro- diagnostic use)

The buffer used was bovine gamma globulin in phosphate buffer, with 0.1% sodium azide as preservative. (Stored at 15-30° c)

2. Apparatus

2.1 Automated Fluorescence Polarization Analyzer (Diagnostic Division, Abbott laboratories, Inc., Irving, Tx, U.S.A.)
2.2 Centrifuge (Diagnostic Division, Abbott Laboratories, Inc., Irving, Tx, U.S.A.)
2.3 Centrifuge (Model cs., Internal (ICE) Centrifuge Internation Equipment, Inc., Needham His, Mass, U.S.A.)
2.4 Freezer (Forma Bio-freezer, Forma Scientific, Inc, U.S.A.)

METHOD

1. Subjects

The subjects studied were Thai-inpatients of Surgical Department of Police General Hospital and were decided to give gentamicin by physicians. The patients included in the study were either with normal renal function or renal impairment. All patients were treated

with gentamicin by traditional prescribing practices of the physician. When patients were admitted, laboratory data such as serum creatinine (Scr), blood urea-nitrogen (BUN), white blood cell count (WBC), culture and sensitivity (C&S) were ordered by the physician. All of patient data related to the studies were recorded such as age, sex, weight, height, medical history, diagnosis, dosage regimen, duration of gentamicin therapy, gentamicin serum concentration and other laboratory data.

2. Dosage Regimen and Administration

Dosage regimen of gentamicin prescribed by physician in general practice in Surgical Department of Police General Hospital, is commonly 60 or 80 mg every 6 or 8 hours by IM, IV intermittant infusion and IV push. For IV infusion, the calculated amount of gentamicin was diluted with 50 ml of intravenous solution before infusion at a constant rate for 30 minutes.

3. Sample Collection

The gentamicin serum concentration was considered to be at steady state after the fixed dosage regimens of the drug were given to the patients for at least 4-5 half-lives. Here, the samples were collected after the drug was given for at least 2 days. Therefore on the third day, each of blood samples was drawn 5 ml from forearm of patient to determine concentrations.Blood samples for determination of trough concentration level

should be drawn immediately before the next dose. Blood samples for determination of peak concentration were drawn at 30, 90 minutes after complete IV infusion and IM injection respectively. For IV push, three blood samples were collected.The trough and the peak concentrations were drawn immediately , before and after the drug was given respectively. The third sample (Cpost) was drawn at 60 minutes after drug administration.

If the blood samples were not drawn at the right times as described above, the actual times of blood drawn were recorded instead. The blood samples must be drawn from another arm which drug was not administered.

All the blood samples were kept in glass tubes. After clotting , the serum was separated by centrifugation (3000 rpm for 10-15 minutes at room temperature) and were assayed by fluorescence polarization immunoassay (TDx^{R} Analyzer System). All the samples were assayed within the same day that the samples were collected.

4. Analytical Method

Gentamicin serum levels were determined by Immunoassay Method using TDx^{R} Analyzer System.

4.1 Performing an Assay Calibration

The required items were calibration carousel, cuvettes, sample cartridge, reagent pack, calibrators and controls.

4.1.1 Preparation of the carousel

- Load the carousel with 15 cuvettes in positions #1 to #15
- Load the carousel with 15 sample cartridges in positions # 1 to # 15
- Pipette at least 50 µl of calibrators to the sample wells as follows :

Calibrator A in wells 1 and 2,

Calibrator B in wells 3 and 4,

Calibrator C in wells 5 and 6,

Calibrator E in wells 7 and 8,

Calibrator F in wells 9 and 10,

Control L in well 13,

Control M in well 14 and

Control H in well 15

(Note : Gently invert reagent pack, calibrator pack and control pack 3 times before use)

4.1.2 Load the carousel in the instrument

4.1.3 Load the reagent pack in the instrument

4.1.4 Close the door of the TDx^{R} analyzer

4.1.5 Press run

4.1.6 The instrument commences

4.1.7 Wait for run to complete

4.1.8 Keep to printout

4.2 Performing an assay run

Items required were assay carousel, cuvettes sample cartridges and reagent pack.

4.2.1 Preparation of the carousel

- Load the carousel with 2 cuvettes in positions # 1 to # 2 (For 2 specimens)
- Load the carousel with 2 sample cartridges in positions # 1 to # 2
- Pipette at least 50 µl of specimens in the sample wells as follow :
  Specimen # 1 in well # 1 and
  Specimen # 2 in well # 2

4.2.2 Load the carousel in the instrument

4.2.3 Load the reagent pack in the instrument

4.2.4 Close the door of the TDx^{R} analyzer

4.2.5 Press run

4.2.6 The instrument commences operation

4.2.7 Wait for run to complete

4.2.8 Keep the printout

5. Monitoring Serum Gentamicin Levels

5.1 Serum Gentamicin Concentration Evaluation

Gentamicin concentrations were evaluated whether both peak and trough concentrations were within therapeutic range. (Acceptable gentamicin therapeutic peak levels were within 4 to 8 µg/ml while acceptable range for trough concentrations were 1 to less than 2 µg/ml) If gentamicin concentrations were not within therapeutic range, the drug dosage regimen was adjusted and followed up until the desired therapeutic ranges were achieved. In some patients whom the IV push gentamicin were given, the administration route was changed to be IV infusion in the same dosage regimen.

5.2 Calculation of Dosage Regimen by Pharmacokinetic Method

5.2.1 Determinination of Pharmacokinetic Parameters

For IV infusion, the pharmacokinetic parameters were calculated by using equation 9 (see Appendix A) and for IM and IV push, equation 7 and 8 (see Appendix A) were used respectively. As Vd of patients recieved gentamicin by IM and IV push were not able to calculate from serum concentration, therefore, mean population Vd from literature was used (Hull, Sarubbi and Hill, 1976).

5.2.2 Calculation of Dosage Regimen Using Individual Pharmacokinetic Parameters.

Dosage regimen was calculated by using individual pharmacokinetic parameters of patient. Selected desirable peak (4-8 µg/ml) and trough (1-2 µg/ml) concentrations were used to calculate dosing interval from equation 10 (Appendix A), The calculated dosing interval was rounded up and adjusted to a figure convenient for dosage administration.

Maintenance doses were calculated using the adjusted dosing interval by equation 13 or 14 (Appendix A). Predictions of steady-state peak and trough levels were calculated by equation 11 and 12 (Appendix A). Proper dosage regimen was selected to give desired peak and trough concentrations.

Calculated dosage regimen was rounded up and adjusted to a figure convenient for administration.

5.3 Dosage Administration

The patients whose gentamicin levels were not within therapeutic range, the dosage regimen was then adjusted and given by intravenous infusion for 30 minutes. The dosage regimen was calculated as deseribed in 5.2.

5.4 Sample Collection

After the patients were on a fixed dosage regimen (as described in 5.2) of gentamicin for at least one day, two blood samples were drawn to determine peak and trough concentration.

6. Data Analysis

6.1 Serum gentamicin concentrations of patients treated by traditional practice and pharmacokinetic method were analysed to see which of them would give better serum gentamicin concentrations within therapeutic range.

Percentage of patients whose serum gentamicin concentrations was within therapeutic,subtherapeutic and overtherapeutic ranges were compared between traditional and pharmacokinetic method, between intramuscular and intravenous infusion. Serum gentamicin concentrations were measured and considered if peak and/or trough were within therapeutic range. Peak level of greater than 8 µg/ml and trough level of more than 2 µg/ml were considered overtherapeutic range. Subtherapeutic range was considered when peak and trough level less than 4, 1 µg/ml respectively.

6.2 Comparison of different methods for predicting serum gentamicin concentrations.

Kel and Vd were calculated by using different method to predict gentamicin blood levels.

The different elimination rate constants were calculated as follows :

1. Kel $(Scr)_P$ in equation 5 (Appendix A) was calculated by using estimated creatinine clearance (CrCl) from serum creatinine(Scr) by Cockcroff and Gualt's method (1976). CrCl was calculated from equation 1-4 (Appendix A).

2. Kel (blood) in equation 7,8 and 9 (Appendix A) was calculated by using two serum gentamicin concentrations.

3. Kel $(Scr)_T$ in equation 6(Appendix A) was calculated using data of the first 20 patients to determine slope and intercept of regression line between Kel (blood) and CrCl.

The different volume of distributions were calculated as follows :

1. Vd (blood) in equation 9 was calculated by using two serum concentration.

2. Vd (mean) in equation 5,6,7 and 8 was calculated by using mean population Vd from literature.

The predicted gentamicin blood levels were calculated by using four different methods as follows :

1. Using Kel(Scr)$_F$ and Vd(mean) in equation 5 (Appendix A)
2. Using Kel(Scr)$_T$ and Vd(mean) in equation 6 (Appendix A)
3. Using Kel(blood) and Vd(mean) in equation 7 or 8 (Appendix A)
4. Using Kel(blood) and Vd(blood) in equation 9 (Appendix A)

The calculated peak and trough concentrations were compared with those measured values.

Statistical Method

The method of Sheiner and Beal (1981)was used to analyze the predictions. For each of the different methods, measured serum concentrations to give prediction error. Squared prediction errors were then determined. The different methods were evaluated for bias and precission. By calculating the 95% confidence intervals for the mean prediction error and the mean squared prediction error. Analysis of variance of the mean prediction error and the mean squared prediction error for each method was performed. The example for calculating the absolute and relative performance were shown in Appendix B.