CHAPTER III

MATERIALS AND METHODS

Materials

1. TDx Theophylline Monoclonal II

1.1 No. 8A53.01, Theophylline monoclonal II calibrators

Six vials of accurately measured amounts of theophylline in human serum at the following concentrations :

vial	Theophylline concentration (mcg/ml)
A	0.0
B	2.5
C	5.0
D	10.0
E	20.0
F	40.0

Preservative : 0.1% Sodium azide

1.2 No. 8A53.10, Theophylline monoclonal II controls

Three vials of theophylline in human serum should read within the following ranges :

vial	Theophylline concentration (mcg/ml)
L	6.30 - 7.70
M	10.80 - 13.20
H	23.40 - 28.60

Preservative : 0.1% Sodium azide

1.3 No. 8A53.60, Theophylline monoclonal II reagent pack

The reagent pack consists of three vials

S < 0.05% Theophylline antibody (mouse monoclonal) in buffer containing surfactant and protein stabilizer (0.4 ml)

Preservative : 0.1% Sodium azide

T < 0.01% Theophylline fluorescein tracer in buffer with protein stabilizer (0.4 ml)

Preservative : 0.1% Sodium azide

P Pretreatmet solution. Protein stabilizer in buffer (0.4 ml)

Preservative : 0.1% Sodium azide

1.4 No. 9519.02, Buffer solution

The buffer solution contains 0.1 M phosphate buffer.

Preservative : 0.1% Sodium azide

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 Freezer (Forma Bio-freezer, Forma Scientific, Inc, U.S.A.)

Methods

1. Subjects

Sujects Selection :

The subjects of this study were preterm infants* admitted to Queen Sirikit National Institute of Child Health and they had not ever used theophylline before the study. They were enrolled in the study if they had initially received intravenous aminophylline (Theophylline salt) loading dose. Indication for theophylline used in this study was not limited.

* Preterm infants were defined to the infants who were born before 37 weeks' gestation age.

Sample Size : At least 30 cases of preterm infants who received theophylline therapy.

All of available patients data related to the study were recorded such as sex, gestation age, apgar score at 1 minute and 5 minutes, major diagnosis during theophylline therapy, age and weight at the beginning of theophylline treatment, indication for theophylline treatment, factors affected on theophylline pharmacokinetics, laboratories and clinical data.

2. Dosage Regimen and Administration

Dosage regimen of theophylline in preterm patients was traditional dosage regimen prescribed by the physician at Queen Sirikit National Institute of Child Heath. It was commonly prescribed as 4 - 6 mg/kg intravenous aminophylline loading dose (approximately 5 mg/kg), followed by 1 - 2 mg/kg intravenous aminophylline maintenance dose every 8 - 12 hours. The maintenance dose was given 12 hours after intravenous aminophylline loading dose. All doses were infused over 10 - 15 minutes.

3. Sample Collection

Non-steady state Sample Collection

Theophylline serum concentration were collected at the 6th and 12th hour after the loading dose which the theophylline serum concentration at the 12th hour must be collected before initiation the maintenance dose. The correct time was recorded when theophylline serum concentration was not collect at that times.

Steady State Sample Collection

Steady state condition was defined as that point at which the patients had received a constant dose and dosing interval of the maintenance dose at least 3 half lives of theophylline in preterm infants. So that, the steady state theophylline serum concentrations in this study were collected at least 4 days after the constant dose and dosing interval. During steady state, the blood samples were collected for trough concentrations, the 8th hour post dose when theophylline was given every 8 hours or the 12th hour post dose when theophylline was given every 12 hours or promptly before the next dose. The correct time for collection blood samples during steady state was recorded the same as the non-steady state blood samples.

The amount of blood sample collected each time was 1 ml. All blood samples were kept in 1.5 ml polypropylene plastic tubes. The serum samples were separated immediately by centrifugation (3000 rpm for 15 minutes) at room temperature after clotting and they were assayed by fluorescence polarization immunoassay (TDx^{R} Analyzer System, Abbott). If the serum sample were not assayed, they were frozen in the freezer until assayed within 24 - 48 hours.

4. Theophylline Therapeutic Monitoring

All patients treated with theophylline were monitored theophylline serum concentrations after the loading dose which called the non-steady state theophylline serum concentrations. The two points, non-steady state theophylline serum concentration were used to calculate individual patients pharmacokinetic parameters (equation 1, 2, and 3.2 in appendix III) and calculate the predicted trough theophylline serum

concentrations during steady state (equation 5.2) based on the initial maintenance dose ordered by the physicians. The new maintenance doses were recommended when the predicted trough theophylline serum concentrations were in subtherapeutic range (< 6 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or < 10 mcg/ml for the patients used theophylline for BPD) or overtherapeutic range (> 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or > 20 mcg/ml for the patients used theophylline for BPD). In the patients whose traditional maintenance dose was adjusted, the target level was 4 mcg/ml or higher which was lower than the usual therapeutic range in order to play safe, since many uncertainly including the pharmacokinetic parameters could happen in these patients. In addition, some previous studies had reported that at the level of 4 – 5 mcg/ml theophylline could prevent the apnea in the preterm infants. Nevertheless, the therapeutic range for treatment of apnea of prematurity in most studies was 6 – 12 mcg/ml. If the maintenance dose which produced the predicted steady sate serum concentration equal to 4 mcg/ml was higher than the maximal maintenance dose recommended in clinical practice (6 mg/kg/day), the new maintenance dose would be recommend as the maximal dose (6 mcg/kg/day) and the predicted steady state serum concentration would be less than 4 mcg/ml. However, dosage regimen adjustment was based on the physician decision. If the predicted trough theophylline serum concentration was within therapeutic range, the initial maintenance dose prescribed by the physician would continue.

Together with theophylline serum concentration monitoring, the patients were monitored the clinical responses both benefit effects and adverse reactions. The benefit effects and adverse reactions were assessed as follow :

Benefit effect

1. Absent of apnea within 5 day after theophylline treatment or dose adjustment for the patients used theophylline for apnea.
2. Successful extubation and no apnea occurred for the patients used theophylline as an adjuvant to weaning
3. Decreasing of dyspnea for the patients used theophylline for BPD.

Adverse reaction

1. Gastrointestinal system : vomiting, feed intolerance, and etc.
2. Central nervous system : tremor/agitaion, seizure, and etc.
3. Cardiovascular system : tachycardia, arrhythmia, and etc.
4. The others : hyperglycemia and etc.

Naranjo 's Algorithm was used to assess theophylline adverse reactions and the study would report only definite and possible adverse reactions of theophylline.

5. Analytical Method

Theophylline serum concentration were determined by immunoassay method using TDx^{R} Analyzer System, Abbott Labolatories based on fluorescence polarization technique.

5.1 Prefoming an Assay Calibration

The equipments consisted of calibration carousel, cuvettes, sample cartridges, reagent pack, calibrators and controls. The calibration method was done as follow :

1. Loaded the sample cartridges and cuvettes in loc 1 to 9 of the carousel labelled with " CAL" , locked carousel.
2. Mixed calibrators A to F approximately 3 - 5 times and pipetted 100 µl of calibrators into the 1st to the 6th sample well.
3. Mixed control L, M, and H approximately 3 - 5 times and pipetted 100 µl of control into the 7th to 9th sample well.
4. Mixed the reagents approximately 3 - 5 times, opened the covers of the reagents and loaded the reagent pack in the instrument.
5. Loaded the carousel in the instrument.
6. Closed the door
7. Pressed run.

* Remark : Made sure that the air bubbles were not happened when the calibrator, controls and reagents were mixed.

An acceptable theophylline monoclonal II asay calibration curve should meet the following criteria :

1. Polarization error (PERR) was -2.00 to +2.00 for all calibrators
2. Root mean square (RMSE) was less than or equal to 1.00.
3. All controls were within the acceptable ranges.

5.2 Performing an Assay Run

The equipments consisted of assay carousel, sample cartridges, and reagent pack. The method for performing assay run was done as follow :

1. Loaded the sample cartridges and cuvettes in loc 1, 2,... of the carousel, locked carousel.
2. Pippetted at least 50 μl of serum sample in the sample well as follow : the 1^{st} , 2^{nd},... serum sample in the sample well in loc 1, 2,..... , respectively.
3. Mixed the reagents approximately 3 - 5 times, opened the covers of the reagents and loaded the reagent pack in the instrument.
4. Loaded the carousel in the instrument.
5. Closed the door.
6. Pressed run.

6. Data Analysis

6.1 Theophylline Serum Concentrations during Non-steady state

Percentage of the patients had theophylline serum concentration after loading dose within subtherpeutic range (< 6 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or < 10 mcg/ml for the patients used theophylline for BPD), therapeutic range (6 - 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or 10 - 20 mcg/ml for the patients used theophylline for BPD), and overtherapeutic range (> 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or > 20 mcg/ml for the patients used theophylline for BPD).

6.2 Theophylline Serum Concentrations during Steady State

Percentage of the patients had theophylline serum concentration during steady state within subtherpeutic range (< 6 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning < 10 mcg/ml for the patients used theophylline for BPD), therapeutic range (6 - 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or 10 - 20 mcg/ml for the patients used theophylline for BPD), and overtherapeutic range (> 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or > 20 mcg/ml for the patients used theophylline for BPD).

6.3 Comparison between the Measured and the Predicted Theophylline Serum Concentration during Steady State

The reliability the prediction of theophylline serum concentrations during steady state based on the drug concentrations during non-steady state was analysed by using correlation analysis with significant level at 0.05.

The precision of prediction of theophylline serum concentration during steady state based on the drug concentrations during non-steady state was analysed by calculation of the root mean square prediction error (RMSE) value with 95% confidence interval.

6.4 Correlation between Theophylline Serum Concentration and Clinical Response

Percentage of the patients had benefit effect or adverse reaction during theophylline serum concentration was within subtherapeutic range (< 6 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or < 10 mcg/ml for the patients used theophylline for BPD), therapeutic range (6 - 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or 10 - 20 mcg/ml for the patients used theophylline for BPD), and overtherapeutic range (> 12 mcg/ml for the patients used theophylline for apnea and used theophylline as an adjuvant to weaning or > 20 mcg/ml for the patients used theophylline for BPD).