CHAPTER III

MATERIALS AND METHODS

Materials

1. TDx^{R} Theophylline II

1.1 No. 9517 - 01, Theophylline 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. 9517 - 10, Theophylline Controls :

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

Vial	Theophylline Concentration (mcg/ml)
L	6.3 - 7.7
M	10.8 - 13.2
H	23.4 - 28.6

Preservative : 0.1% Sodium Azide

1.3 No. 9517 - 60, Theophylline II Reagent Pack

The Reagent Pack contains the following ready - to use reagents (3 ml in each vial) :

Vial	Components
P	Pretreatment Solution
S	< 25% Theophylline Antiserum (Mouse monoclonal) in buffer with protein stabilizer
T	< 0.01% Theophylline Fluorescein tracer in buffer containing surfactant

Preservative : 0.1% Sodium Azide

1.4 No. 9519 - 05 : Dilution Buffer

The dilution buffer contains 0.1M Phosphate buffer and 0.1% Sodium Aazide as a preservative

2. Apparatus

2.1 Automated Fluorescence Polarization Analyzer (Diagnostic Division, Abbott Laboratories, Inc., Irving, TX, USA)

2.2 Centrifuge (H - 103N Series, Kokusan, Biomed Group Co.,Ltd.)

2.3 Freezer (SR - MC - 1, No frost, Hitachi Co.,Ltd.)

Methods

1. Subjects

Patient Selection : Thai patients with respiratory disorders admitted to Medical Department at Ratchaburi Hospital. Patients included in this study were not critically ill. All patients were treated with theophylline alone or theophylline together with other drugs (beta - adrenergic agonists, corticosteroids and anticholinergic drugs) by traditional physician prescribing practices. Age of the patients were equal to or greater than 20 years.

Sample Size : At least 60 patients were treated with theophylline alone or theophylline together with other drugs.

All of available patients' data related to the study were recorded; including age, gender, weight, height, medical history, smoking history, diagnosis, drugs administered, dosage regimens, duration of theophylline therapy, factor affecting theophylline elimination, clinical responses to theophylline therapy in the patients treated with theophylline alone and theophylline together with other drugs, and other clinical and laboratory data.

2. Dosage Regimen and Administration

Dosage regimen of theophylline and other drugs were recommended by physicians prescribing in general practice of Medical Department at Ratchaburi Hospital. Dosage regimen of theophylline was based upon the following :

2.1 Intravenous Infusion for 15 minutes every 6 hours : Dosage of aminophylline was 800 - 1000 mg/day depending on the severity of respiratory disorder symptoms in the patients.

2.2 Oral sustained release dosage form : Dosage of theophylline was 8 to 10 mg/day/kg depending on the severity of respiratory disorder symptoms in the patients. Range of dosage of theophylline was 200 - 500 mg/day which taken once or twice daily (dosing interval was 12 or 24 hours).

Note : Theophylline preparations used for respiratory disorder treatment at Ratchaburi Hospital as the following dosage forms:

1. Injection preparation was Aminophylline (Atlantic).

2. Oral sustained release preparations were Theo - Dur^{R} (Astra), Nuelin^{R} SR (3M Pharm), and Theo - 24^{R} (Searle).

3. Blood Sample Collection

Theophylline serum concentration was considered to achieve steady state after the fixed dosage regimens of the drug were given to the patients for at least two days. Blood sample for determination of serum theophylline concentration was drawn (3ml) from forearm of patient at the following times :

1. Intravenous Infusion for 15 minuts every 6 hours : Peak serum concentration was obtained at one hour after finished infusion. Trough serum concentration was obtained immediately before the next dose. (Winter, 1993).

2. Theo - Dur^{R} and Nuelin^{R} SR (dosing interval was 12 hours), Theo - 24^{R} (dosing interval was 24 hours) : Peak or average serum concentration was obtained at midpoint of dosing interval. Trough serum concentration was obtained immediately before the next dose. (Barnhart et al., 1988; Blackbourn and Sunderland, 1987; Hendeles et al., 1984; Hendeles et al., 1986; Peck et al., 1991; Winter 1992).

Blood samples were allowed to clot and centrifuged immediately (2,000 rpm for 10 minutes at room temperature). Serum was separated and frozen until assayed. Usually, serum samples were assayed by fluorescence polarization immunoassay (TDX^{R} Analyzer System) within 24 - 48 hours.

4. Therapeutic Monitoring of Theophylline

All patients treated with theophylline were monitored for theophylline serum levels and clinical responses to theophylline therapy. Clinical responses of the patients were assessed as follow :

1. Beneficial Effects of Theophylline :
   - 1.1 Decreasing of wheeze, dyspnea, cough, rales, mucus and other symptoms.
   - 1.2 Increasing of peak expiratory flow rate value

2. Adverse Reactions of theophylline :
   - 2.1 Gastrointestinal system and nervous system effects
   - 2.2 Cardiovascular system effects

Example of record form for patient's data and theophylline serum concentration measurement, assessment of clinical responses in patient treated with theophylline were demonstrated in Appendix I

Recommend physicians to adjust appropriate theophylline dosage regimen for individual patient showed inappropriate clinical response (e.g., no beneficial effect from theophylline and/or theophylline adverse reaction occuring) using pharmacokinetic theories. Application of pharmacokinetic theories to adjust for the individual appropriate theophylline dosage regimen based on serum level (determination from peak serum concentration) and clinical response in the patient. Therapeutic range from literatures was 10 to 20 mcg/ml. Equations from pharmacokinetic theories were used to calculate appropriate theophylline dosage regimen for individual patient were as follow : (Peck et al., 1991; Winter, 1992; 1993).

1. For intravenous infusion for fifteen minutes every 6 hours of aminophylline, the pharmacokinetic parameters and serum concentration at steady state were calculated using equations 1 to 3, 5 to 6 and 9 (Appendix II).

2. For oral sustained release preparations, the pharmacokinetic parameters and average serum concentration at steady state were calculated using equations 1 to 3, and 6 to 8 (Appendix II).

5. Comparison between the measured and predicted theophylline serum concentrations.

5.1 For intravenous infusion for fifteen minutes every 6 hours of aminophylline, the predicted theophylline serum concentration was calculated from equations 1 to 5 and 9 (Appendix II).

5.2 For oral sustained release preparations, the predicted theophylline serum concentration was calculated from equations 1 to 4, and 7 (Appendix II).

6. Pharmacokinetic parameters of theophylline in patients were calculated from equations as follow :

6.1 Intraveneous infusion fifteen minutes every six hours of aminophylline : Volume of Distribution was calculated from equations 1 to 3, Clearance was calculated from equations 5 and 9, Half - life was calculated from equations 1 to 3, 5 to 6 and 9 (Appendix II).

6.2 Oral sustained release preparations : Volume of Distribution was calculated from equations 1 to 3, Clearance was calculated from equation 7, Half - life was calculated from equations 1 to 3, and 6 to 7 (Appendix II).

7. Analytical Method

Theophylline levels in serum samples were determined by immunoassay method using Fluorescence Polarization Technique (TDX^R Analyzer System, Abbott Laboratories)

7.1 Performed an assay calibration

Items required are Calibration Carousel, Cuvettes, Sample Cartridge, Reagent Pack, Calibrators and Controls.

7.1.1 Prepared the carousel

- Loaded the carousel with 15 cuvettes in positions # 1 to # 15
- Loaded the carousel with 15 sample cartridges in positions # 1 to # 15
- Pipetted at least 50 ul of calibrators in the sample wells as follows:
  Calibrators A in wells 1 and 2 ,
  Calibrators B in wells 3 and 4 ,

Calibrators C in wells 5 and 6 ,
Calibrators D in wells 7 and 8 ,
Calibrators E in wells 9 and 10 ,
Calibrators F in wells 11 and 12 ,
Control L in well 13,
Control M in well 14 and
Control H in well 15

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

7.1.2 Loaded the carousel in the instrument

7.1.3 Loaded the reagent pack in the instrument

7.1.4 Closed the door

7.1.5 Pressed RUN

7.1.6 The instrument commenced operation

7.1.7 Waited for run to complete

7.1.8 Keeped the printout for later discussion

7.2 Performed an assay run

Items required are Assay Carousel, Cuvettes, Sample Cartridges and Reagent Pack.

7.2.1 Prepared the carousel

- Loaded the carousel with 3 cuvettes in position # 1 to # 3 (For 3 specimens).
- Loaded the carousel with 3 sample cartridges in position # 1 to # 3
- Pipetted at least 50 ul of specimens in the sample wells as follows :

specimen # 1 in well # 1
specimen # 2 in well # 2 and
specimen # 3 in well # 3.

7.2.2 Loaded the carousel in the instrument

7.2.3 Loaded the reagent pack in the instrument

7.2.4 Closed the door

7.2.5 Pressed RUN

7.2.6 The instrument commenced operation

7.2.7 Waited for run to complete

7.2.8 Keeped the printout for later discussion

8. Data Analysis

8.1 Therapeutic Monitoring of Theophylline

1. Percentage of patients had theophylline serum concentration within subtherapeutic range (< 10 mcg/ml), therapeutic range (10 to 20 mcg/ml), overtherapeutic range (> 20 mcg/ml). Incidence of no beneficial effect and adverse reactions of theophylline.

2. Percentage of patients showed beneficial effects while theophylline serum concentrations within subtherapeutic, therapeutic, and overtherapeutic ranges, Percentage of patients showed adverse reactions of theophylline while theophylline serum concentrations within subtherapeutic, therapeutic and overtherapeutic ranges.

3. Percentage of patients required theophylline dosage regimen adjustment. Percentage of patients showed improvement in clinical response after theophylline dosage regimen adjustment

8.2 Comparison between the measured and predicted theophylline serum concentrations .

1. Percentage of difference between the measured and predicted values.

2. Mean ± SD of Percentage of difference between the measured and predicted values.

3. Percent coefficient of variation (%cv) between the measured and predictied values.

8.3 Pharmacokinetic parameters of theophylline in Thai patients.

1. Mean ± SD of clearance, and half - life in the patients with various clinical situations.

2. Comparison of the mean clearance between group of patients using Unpaired Student's t - test.

3. Relationship between theophylline clearance and the age of patients using Linear Regression.