CHAPTER III

MATERIALS AND METHODS

Materials

1. TDx[R] Phenytoin

1.1 No. 9507-01, Phenytoin Calibrators:

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

Vial	Phenytoin Concentration (μg/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. 9507-10, Phenytoin Controls:

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

Vial	Phenytoin Concentration (μg/mL)
L	6.75 - 8.25
M	13.5 - 16.5
H	27.0 - 33.0

Preservative: 0.1% Sodium Azide

1.3 No. 9507-20, Phenytoin Reagent Pack

The phenytoin reagents consist of the following:

Vial	Components
P	Pretreatment Solution. Surfactant in buffer containing protein stabilizer.
S	< 1% Phenytoin Antiserum (Sheep) in buffer with protein stabilizer.
T	< 0.01% Phenytoin fluorescein tracer in buffer containing surfactant and protein stabilizer.

Preservatives: 0.1% Sodium Azide

1.4 No. 9519-05 : Dilution Buffer

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

2. Apparatus

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

2.2 Centrifuge KOKUSAN H-103N Series

2.3 Freezer

Methods

1. Subjects

Patient Selection : Epileptic Thai outpatients and inpatients came at Prasat Neurological Institute whom was treated with phenytoin alone or phenytoin together with other antiepileptic drugs (phenobarbital, carbamazepine, valproic acid and benzodiazepines). Patients

Patients included in this study were not critically ill and they were tonic-clonic seizure. Patients who not controlled seizure and/or had adverse drug reactions were selected. However, patients who controlled seizure and not observed adverse drug reactions were randomly selected in this study.

Sample Size : At least 50 patients.

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

2. Dosage Regimen and Administration

The usual dosage regimen of phenytoin prescribed for patients at Prasat Neurological Institute are as follow:

2.1 Sodium phenytoin injection (50 mg./mL) composes of 92% phenytoin is used for the treatment of status epilepticus. In adults, a loading dose of 10-15 mg/kg, dilute in normal saline, should be administered slowly via IV route at a rate not exceeding 50 mg/min.

2.2 Sodium phenytoin capsule 100 mg composes of 92% phenytoin and Phenytoin tablet composes of 100% phenytoin. The loading dose and maintenance dose is usually calculated according to the bodyweight of the patients. The recommended loading dose is 15 to 20 mg/kg and the recommended maintenance dose is 5 to 7 mg/kg/d.

Note : Phenytoin preparations available at Prasat Neurological Institute are:

1. Injection preparation was Dilantin ® (Parke-Davis).

2. Oral preparation were Dilantin capsule 100 mg. and Dilantin infatab 50 mg. (Parke-Davis).

3. Blood Sample Collection

Phenytoin serum concentration was considered to acheive steady state after the fixed dosage of the drug were given to the patients for at least seven days. Five milliliters blood sample was drawn from forearm of the patient for determination of serum phenytoin concentration.

Steady state was considered seven days after administerd phenytoin. The blood sample usually was drawn 1 hour before the administration of phenytoin which was taken after breakfast and more than 9 hours after the administration of phenytoin at bedtime.

Blood samples were allowed to clot and centrifuged immediately (5,000 rpm for 5 minutes at room temperature). Serum was separated and frozen until being assayed by fluorescene polarization immunoassay (TDX^{R} Analyzer System) within 24-48 hours.

In order to assess the correlation between the serum and the saliva phenytoin concentrations, one milliliter of saliva sample was collected in several patients at the same time when the blood sample was collected. These samples were centrifuged at 1,000 rpm/min for 5 minutes. The supernatant was then separated and frozen until being assayed which were usually done within 24-48 hours.

4. Therapeutic Monitoring of Phenytoin

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

1. Beneficial Effects of Phenytoin:

Absolutely : No seizure was observed after phenytoin serum concentration acheived to steady state.

Partially : The duration, severity and frequency of seizure in the patients was decreased for more than 50% after phenytoin serum concentration steady state.

2. Adverse Reactions of Phenytoin:

2.1 Nervous System Effects

2.2 Gastrointestinal System Effects

2.3 Others

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

In the individual patient who showed inappropriate clinical response (e.g., no beneficial effect from phenytoin , partially seizure control and/or phenytoin adverse reaction occuring). A more appropriate phenytoin dosage regimen, using pharmacokinetic theories was recommended to the physician. The new dosage regimen was administered to the patient and the clinical response was again observed, blood samples were collected and assayed for phenytoin serum concentrations.

The pharmacokinetic theories applied for adjustment of the individual appropriate phenytoin dosage regimen were based on serum level (determination from trough serum concentration) and clinical response of the patient. The theoritical therapeutic serum concentration range of phenytoin was 10 to 20 μg/mL. Equations used to calculate appropriate phenytoin dosage regimen for individual patient were demonstrated in Appendix II.

5. Analytical Method

The concentrations of phenytoin in serum samples were determined by immunoassay method using Fluorescence Polarization Technique (TDX^{R} Analyzer System, Abbott Laboratories).

5.1 Performed an assay calibration

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

5.1.1 Prepared the carousel

- Loaded the carousel with 15 cuvettes in position # 1 to # 15
- Loaded the carousel with 15 sample cartridges in positions # 1 to # 15
- Pipetted at least 50 µl 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 five or more times before use).

5.1.2 Loaded the carousel in the instrument

5.1.3 Loaded the reagent pack in the instrument

5.1.4 Closed the door

5.1.5 Pressed RUN

5.1.6 The instrument commenced operation

5.1.7 Waited for run to complete

5.1.8 Keeped the printout for later discussion

5.2 Performed an assay run

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

5.2.1 Prepared the carousel

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

  specimen # 1 in well # 1

  specimen # 2 in well # 2 and

  specimen # 3 in well # 3.

5.2.2 Loaded the carousel in the instrument

5.2.3 Loaded the reagent pack in the instrument

5.2.4 Closed the door

5.2.5 Pressed RUN

5.2.6 The instrument commenced operation

5.2.7 Waited for run to complete

5.2.8 Keeped the printout for later discussion

6. Prediction of Serum Phenytoin Concentrations from Pharmacokinetic Parameters.

Prediction of serum phenytoin concentrations was calculated by several method as followed (Appendix II):

1. Calaulated by using two population pharmacokinetic parameters (K_m = 4 and V_{max} = 7).
2. Calculated by using Wagner's equation.
3. Calculated by using one population pharmacokinetic parameters that fixing K_m = 4.
4. Calculated by using one population pharmacokinetic parameters that fixing V_{max} = 7.
5. Calculated by using individual pharmacokinetic parameters of each patients.

7. Data Analysis

7.1 Therapeutic Monitoring of Phenytoin

1. Determine the percentage of patients who had phenytoin serum concentration within subtherapeutic range (< 10 μg/mL), therapeutic range (10 to 20 μg/mL), overtherapeutic range (> 20 μg/mL) and incidence of no beneficial effect and adverse reactions of phenytoin.

2. Determine the percentage of patients who showed beneficial effects while their phenytoin serum concentrations were within subtherapeutic, therapeutic, and overtherapeutic ranges, the percentage of patients who showed adverse reactions of phenytoin

while their phenytoin serum concentrations were within subtherapeutic, therapeutic and overtherapeutic ranges.

3. Determine the percentage of patients who required phenytoin dosage regimen adjustment and also the percentage of patients who showed improvement in clinical response after phenytoin dosage regimen adjustment.

7.2 Comparison between the measured and the predicted phenytoin serum concentrations.

1. Calculated the percentage of difference between the measured and predicted values of each individual patient.

2. Calculated the mean ± SD of percentage of difference between the measured and predicted values.

3. Determined the percent coefficient of variation (%CV) between the measured and predicted values.

7.3 Compared the predicted phenytoin concentrations calculated by different methods using comparison the percent coefficient of variation (%CV).

7.4 Compared the pharmacokinetic parameters obtained from population data with those observed individual pharmacokinetic parameters using comparison range.

7.5 Assessed the relationship between serum phenytoin concentration and saliva phenytoin concentration using linear regression.