CHAPTER III

PATIENTS AND METHODS

1. Patients

Gout patients with renal insufficiency who were out-patients of Rheumatology clinic, Rajavithi Hospital during December 2003 to January 2004 were screened into this study. Twenty-seven patients met the inclusion criterias. None of these patients were withdrawn from the study due to the adverse drug reactions, skin rash or AHS.

2. Study design

This study was a before-after experiment design with no control group

3. Subject

Subjects were included into the study based on the follow screening criteria:

Inclusion criteria

Patients who met all of the following criterias were selected into this study:

1. He/she was a gout patient with renal insufficiency (CrCl = 30-60 ml/min) who came to visit the rheumatology clinic at Rajavithi hospital.
2. He/she had not taken any medications which can affect renal functions except for allopurinol, two weeks before initial study or less than fivefold of the half life of the medication before initial study.

3. He/she was willing to be include in this study and signed the patient consent form after receiving the information about this study.

Exclusion criteria

Patients who had at least one of the following criterias were excluded from the study:

1. He/she was hypersensitive to allopurinol.
2. He/she had High blood pressure level according SBP more than 180 mmHg and/or DBP more than 110 mmHg.
3. He/she was required to take : azathioprine, mecaptopurine and cyclophosphamide.
4. He/she had evidence of urinary retention or prostatic hyperplasia.
5. He/she had impairment of liver function: serum glutamic oxaloacetic trancaminase (SGOT) and/or serum glutamic pyruvic transaminase (SGPT) more than two times of normal level (SGOT and/or SGPT > 80 U/L).
6. He/she had 24 hour creatinine clearance which was less than 30 ml/min/1.73m^2
7. He/she was diagnosed from physicians to be inappropriate to enroll in the study.

4. Sample size

The sample size of this study was calculated from this formula

$$N = \frac{(Z_\alpha + Z_\beta)^2 \times Sp^2}{D^2}$$

Where N = number of sample size

Z_α = 1.96 ($\alpha = 0.05$)

Z_β = 1.28 ($\beta = 0.10$)

$Sp^2 = S_1^2 + S_2^2 = 161$ (Rosenfeld et al[44])

$D^2 = 100$ (Briney et al[15])

The parameter of both Sp and D were CrCl (ml/min)

$$N = \frac{(1.96 + 1.28)^2 \times 161}{100}$$

N = 17 (withdrawal 50 %) = 25

5. Step of the study

1. The protocol of this study had been approved by the Ethic Committee of Rajavithi Hospital.

2. Investigator provided complete materials for this study (drugs, instrument and record forms).

3. Patients were included base on screening criteria and then ask to sign inform consent, figure 2 showed the study flow chart.

4. Patients discontinued non-essential medications 2 weeks (or not less than fivefold of the half-life) before study. In some patients who had already received allopurinol treatment before starting of this study, allopurinol should be discontinued for 4 weeks[38].

5. All baseline data of patients, laboratory data, characteristic data and renal function were recorded.

6. Patients received allopurinol (Zyloric®) 300 mg daily for 6 weeks

7. When the patients had completely received allopurinol therapy for 6 weeks:

- Primary and secondary outcomes were assessed.
- Blood samples were collected at before and 5 hours[38,41,42] after taking the last dose of allopurinol and were kept refrigerate at temperature less than -20 °c until analyzed.

- Plasma drug concentrations were analyzed to obtain the minimum concentration (C_{min}), the maximum concentration (C_{max}) respectively.

Table 5 showed the overall schedule of this study and table 6 showed summary of primary and secondary outcomes that were collected in each visit.

Figure 2 : the study flow chart

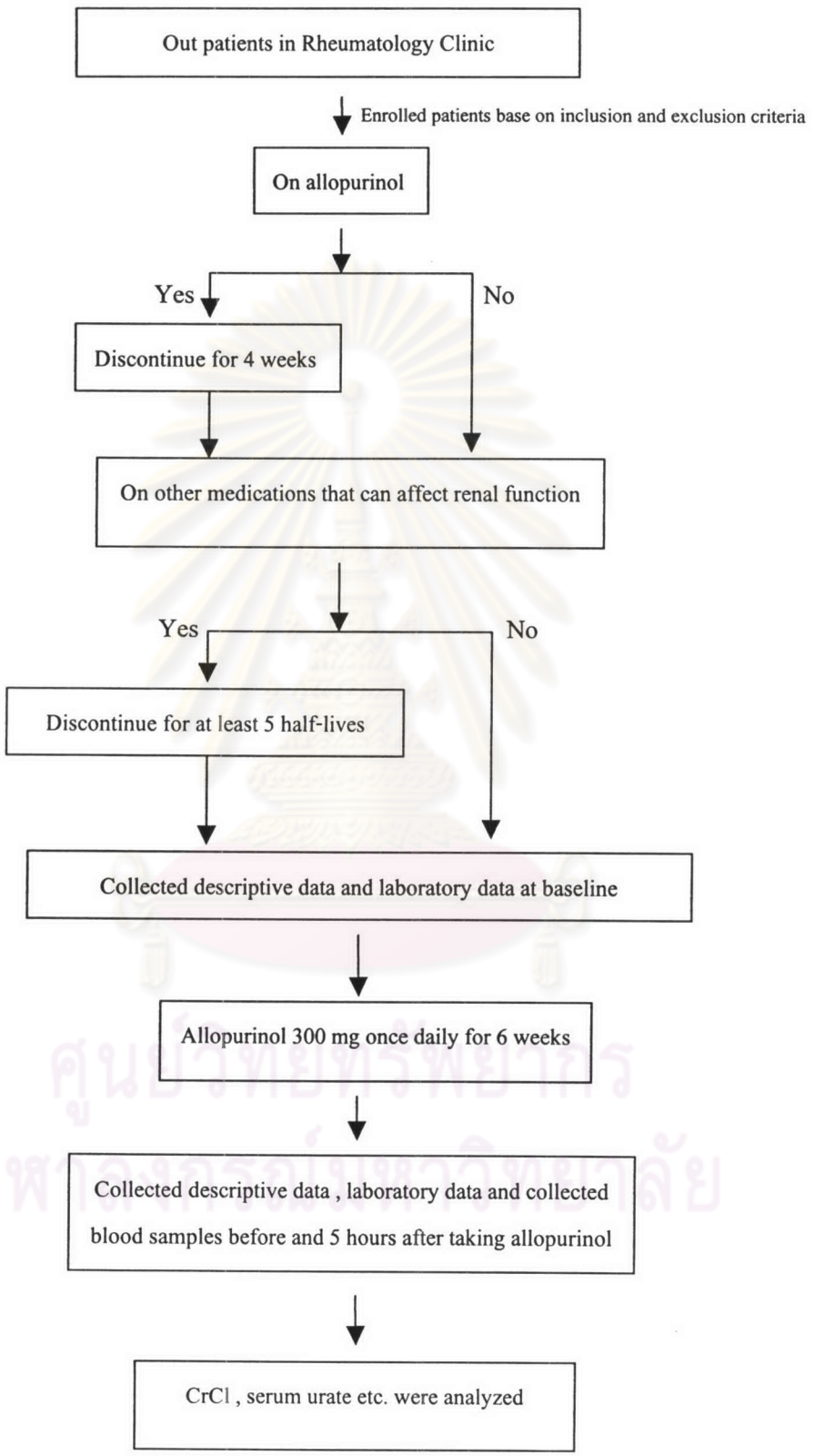

Table 5 : overall schedule of patients in this study

week 0	Visit no. 1	←		enrolled patients and discontinued allopurinol for 4 weeks, other interfering medications for 2 weeks		→
week 2		—			—	—
week 4	Visit no. 2			Allopurinol 300 mg once daily for 6 weeks		
week 6		—			—	—
week 10	Visit no. 3	←		assessed CrCl, serum uric acid, ADRs and collected blood samples		→

Table 6 : summary of primary and secondary outcome collection

visit no.	Demographic data	CBC	LFT	BUN	Cr	Uric acid	BP	Plasma oxypurinol Concentration	Urine 24 hr.			ADRs
									Output	Cr	U/A	
1	/			/	/	/	/					
2	/	/	/	/	/	/	/		/	/		/
3	/	/	/	/	/	/		/	/	/	/	/

CBC = completed blood count

LFT = liver function test

BUN = blood urea nitrogen

Scr = serum creatinine

BP = blood pressure

Cr = creatinine

U/A = urine analysis

6. Drug assays

Concentrations of oxypurinol in plasma samples were quantified using the High Performance Liquid Chromatography (HPLC). Various concentrations of standard oxypurinol , i.e., 0.5, 1, 2, 5, 10, 20 μg/ml were added to blank plasma from normal subject in order to generate standard curve.

Extraction :

The plasma sample extraction method was modified from the methods of Kramer WG et al[45]., Day RO et al[39],and Barthel W et al[41]

50 µl of 8- methylxanthine (4.5µg/50µl) was added to half milliliter of plasma sample in order to use as an internal standard (IS) and the protein was precipitated by adding 0.2 ml 20% trichloroacetic acid (TCA), then mixing by vertex for 1 minute. The sample was centrifuged at 4000 rph for 15 minutes and then a 50 µl aliquot was injected into the HPLC column.

High Performance Liquid Chromatography (HPLC)

The chromatograph consisted of a HPLC pump (Varain®;) a UV detector (Varain® ;) with variable wavelength UV-VIS detector (Varain(®)) set at 254 nm operated at maximum sensitivity of 0.05 a.u.f.t. A Waters reversed-phase column Spherisorb®C_{18}4.6×250 nm ODS was maintained at room temperature. A Waters guard column C_{18} Spherisorb® was connected to the inlet end of the column. The mobile phase composed of acetonitril (ACN) : 0.05 M potassium phosphate (KH_2PO_4) PH 6.0 equal to 2:98 v/v set at the flow-rate of 1 ml/min. In these conditions, oxypurinol and 8-methylxanthine were eluted with retention times of 9.9 and 16.5 minutes, respectively.

Pharmacokinetic parameters

The data composed of 2 concentrations at before taking and at 5 hours after taking allopurinol 300 mg daily this dosage regimen had been taken for at least 6 weeks to assure steady state. The pharmacokinetic parameters of allopurinol were derived, i.e., the minimum plasma oxypurinol concentration at the steady state (C_{min}) , the maxiumum plasma oxypurinol concentration at the steady state (C_{max}) , elimination rate constant (K_a) , volume of distribution (V_d) , the half life ($T_{1/2}$) and clearance of oxypurinol (Cl). Calculation for pharmacokinetic parameters in plasma of individual patient were presented in appendix D.

7. Statistical analysis

Analysis was conducted by using the data analysis software (SPSS for window version 12.0)

Demographic data and baseline characteristic of all patients were presented as descriptive statistic.

Primary outcomes, such as, 24 hour CrCl, serum uric acid level etc. before and after receiving allopurinol treatment were compared using Pair T-test, significant level were set at p-value <0.05. However, if data were not normal distribution, variables were compared by using Wilcoxon sign-rang test (Normality distribution of data was first determined for each variables by using Shapiro-Wilk Test)

The relationship between serum oxypurinol concentration at the steady state and the change of 24 hr-CrCl before and after receiving allopurinol treatment using linear regression. Significant level was set at p value < 0.05

The adverse events after receiving allopurinol treatment were reported to be descriptive characteristic and percent frequency of patients.