CHAPTER III

MATERIALS AND METHODS

Materials

1. Drugs

- Celecoxib 200 mg capsules (Celebrex®) from Pharmacia (Thailand), Ltd.

- Naproxen 250 mg Tablets (Naprosyn LE®) from Roche (Thailand), Ltd.

- Omeprazole 20 mg capsules (O-Sid®) from Siam Pharmaceutical CO., Ltd.

- Pale yellow capsules number 0 from International Capsule CO., Ltd.

2. Instrument

- Mercury Sphygmomanometer (Baumanometer®) from BAUM CO., Ltd

3. Record forms

- Inform consent

- Medication record

- Laboratory record

- 24-hour urine collection form

- Compliance record (Tablet count record)

- Adverse drug reaction profile

- Naranjo's Algorithm

Methods

1. Definitions

- Elderly patients: patients with an age older than 60 years old

- Musculoskeletal problems: musculoskeletal problems such as osteoarthritis, rheumatoid arthritis, crystal induce arthritis, tendinitis

- Underlying diseases: the chronic diseases that concomitant with musculoskeletal problems such as hypertension (HT), diabetic mellitus (DM), coronary artery disease (CAD) and renal insufficiency

- Medications for essential treatments: medications that patients necessarily used for the treatment of underlying diseases

- Risk factors: renal insufficiency, hypertension (HT), diabetic mellitus (DM), coronary artery disease (CAD)

- Normal renal function: $CrCl \geq 60$ ml/min/1.73 m^2

- Renal insufficiency: $CrCl = 30\text{-}60$ ml/min/1.73m^2

- High blood pressure level: systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg

- Primary outcomes: effects of celecoxib and naproxen on renal functions (renal haemodynamic and electrolyte homeostasis) as the follows

    1. Renal haemodynamic

        1.1 Glomerular filtration rate (GFR): assessed creatinine clearance (CrCl) from 24-hour urine collection in accordance with serum creatinine and blood urea nitrogen

        1.2 Blood pressure: systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP)

    2. Electrolyte homeostasis

        2.1 Sodium (mEq/day): assessed sodium excretion in 24- hour urine collection in accordance with serum sodium and edema

        2.2 Potassium (mEq/day): assessed potassium excretion in 24- hour urine collection in accordance with serum potassium

- Secondary outcomes: any adverse drug reaction except renal adverse events that occurred during the study (each adverse event was assessed by using Naranjo's Algorithm)

- Wash out period: the period that patients discontinued either celecoxib or naproxen. Only paracetamol used for relieving pain and inflammation (not more than 4 gm/day). If patients had history of GI complications from NSAIDs, they would receive antiulcer agent (omeprazole 20-40 mg/day) for prevent GI complications (throughout the study)

- Equation used for calculation of the creatinine clearance from

    Equation 1:

    $$CrCl\ (ml/min/1.73m^2) = \frac{Ucr \times V}{Scr \times 1440} \times \frac{1.73\ (m^2)}{BSA\ (m^2)}$$

    where

    V = urine volume (ml/day)

    Ucr = urine creatinine (mg/dl)

    Scr = serum creatinine (mg/dl)

    BSA = body surface area (m^2)

    Equation 2:

    $$BSA\ (m^2) = W^{0.425} \times H^{0.725} \times 0.007184$$

    where

    W = body weight (kg) : H = height (cm)

- Cockcroft-Gault's equation used for calculation of the creatinine clearance from

    Equation 3:

    $$CrCl\ (ml/min)(male) = \frac{(140-age) \times BW}{72 \times Scr}$$

    Equation 4:

    $$CrCl\ (ml/min)(female) = \frac{(140-age) \times BW}{72 \times Scr} \times 0.85$$

    where

    BW = body weight (kg) : Scr = serum creatinine (mg/dl)

If body mass index (BMI) $\geq$ 27 kg/m^2 and/or % ideal body weight (%IBW) $\geq$ 120 used ideal body weight (IBW) instead of actual body weight

Equation 5:

   IBW (kg)(male) = 50 + 2.3 (height in inches-60)

Equation 6:

   IBW (kg)(female) = 45.5 + 2.3 (height in inches-60)

Equation 7:

   %IBW = $\dfrac{\text{actual BW}}{\text{IBW}}$ x 100

Equation 8:

   BMI = W/H^2

   where

      W = body weight (kg) : H = height (m)

• Equation used for calculation of lean body weight (LBW) from

   Equation 9:

      LBW (kg)(male)   =   0.3281W + 0.33929H - 29.5336

   Equation 10:

      LBW (kg)(female) =   0.2957W + 0.41813H - 43.2933

      where

         W = body weight (kg) : H = height (cm)

• Equation used for calculation of the mean arterial blood pressure (MAP) from

   Equation 11:

      MAP = DBP + $\dfrac{(\text{SBP-DBP})}{3}$

      where

         SBP = systolic blood pressure (mmHg)

         DBP = diastolic blood pressure (mmHg)

2. Study design

The design of this study was experimental design: double blind randomized crossover study.

3. Subjects

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

Inclusion criteria

Patients who had completed these criteria were selected in this study

1. Subjects were elderly (more than 60 years old) men or women with musculoskeletal problems and visited in Rheumatology Clinic at Rajavithi Hospital.

2. Patients had never taken any medications, which can affect renal functions before study. Patients received only medications for essential treatments.

3. Patients willing to be included 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 these criteria were excluded from the study

1. Hypersensitivity to sulfonamide and/or nonsteroidal anti-inflammatory drugs

2. History of gastrointestinal bleeding or peptic ulcer (PU) perforate by confirmed at least one of these criteria.

   - stool occult blood positive

   - hemoptysis

   - endoscopy

3. Anemia or malnutrition

4. Serum creatinine level more than 2 mg/dl or creatinine clearance less than 30 ml/min/1.73m^2

5. Having an evidence of diabetic nephropathy, glomerulonephitis, cirrhosis and congestive heart failure (CHF)

6. High blood pressure level according to stage III of JNC VI criteria[117] (SBP more than 180 mmHg and/or DBP more than 110 mmHg)

7. Necessarily use these medications: angiotensin converting enzyme inhibitors (ACEIs), angiotensin II receptor antagonists (AII antagonists), fluconazole and diuretics

8. Having an evidence of urinary retention or prostatic hyperplasia

9. Impaired hepatic function: serum glutamic oxaloacetic transaminase (SGOT) and/or serum glutamic pyruvic transaminase (SGPT) more than two times of normal level (SGOT and/or SGPT > 80U/L)

10. Active systemic diseases which would in the judgment of the physician

4. Sample size

The sample size of this study was calculated from this formula

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

Where

N = number of sample size

$Z_\alpha = 1.96$ ($\alpha = 0.05$)

$Z_\beta = 1.28$ ($\beta = 0.10$)

$S_p^2 = S_1^2 + S_2^2 = 193$ (from the previous study by Rossat et al.[105])

$D^2 = 100$ (from the previous study by Rossat et al.[105])

$$N = \frac{(1.96 + 1.28)^2 \times 193}{100}$$

$N = 21$ (+ withdrawal 50%) = **30**

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 9 showed the study flow chart.

4. Patients discontinued non-essential medications or any NSAID before study for 2 weeks (or not less than fivefold of half-life).

5. All baseline data of patients related to select patients according to criteria such as laboratory data, characteristic data and renal functions.

6. Patients were divided to either sequence I or sequence II after stratified randomization by sex, age, underlying diseases and renal functions as follows.

    6.1 Sequence I: first treatment (naproxen 500 mg twice daily) for 2 weeks and wash out 2 weeks then crossover to second treatment (celecoxib 200 mg twice daily) for 2 weeks and wash out 2 weeks

    6.2 Sequence II: first treatment (celecoxib 200 mg twice daily) for 2 weeks and wash out 2 weeks then crossover to second treatment (naproxen 500 mg twice daily) for 2 weeks and wash out 2 weeks

Investigator provided these medications. Either celecoxib or naproxen was filled in capsules (number 0) to blind both physician and patients.

Table 13 showed overall schedule of patients in this study and Table 14 also showed summary of primary and secondary outcomes that were collected in each visit.

7. Primary and secondary outcomes were assessed in each visit.

8. Patients had at least one of these criteria were withdrawn from the study

    8.1 Poor compliance patients: checked by

        8.1.1 Tablet count: patients who had remain tablets of over or equal to 20% during two continuous visits were excluded from the study

        8.1.2 24-hour urine collection: instructed patients and provided 24-hour urine collection form. Compliance was checked by creatinine in 24-hour urine which kept at 15-20 mg/LBW/day (LBW calculated from equation 9-10). Patients who could not collect 24-hour urine with this following criteria were excluded from the study

    8.2 Occurred adverse events that patients could not tolerate as assessed by physician

    8.3 Change of serum creatinine more than 0.5 mg/dl from baseline

    8.4 Patients with oliguria (urine output less than 400 ml/day)

    8.5 Creatinine clearance less than 30 ml/min/$1.73m^2$

    8.6 Patients changed medication profiles that affect renal functions or lost follow-up throughout the study

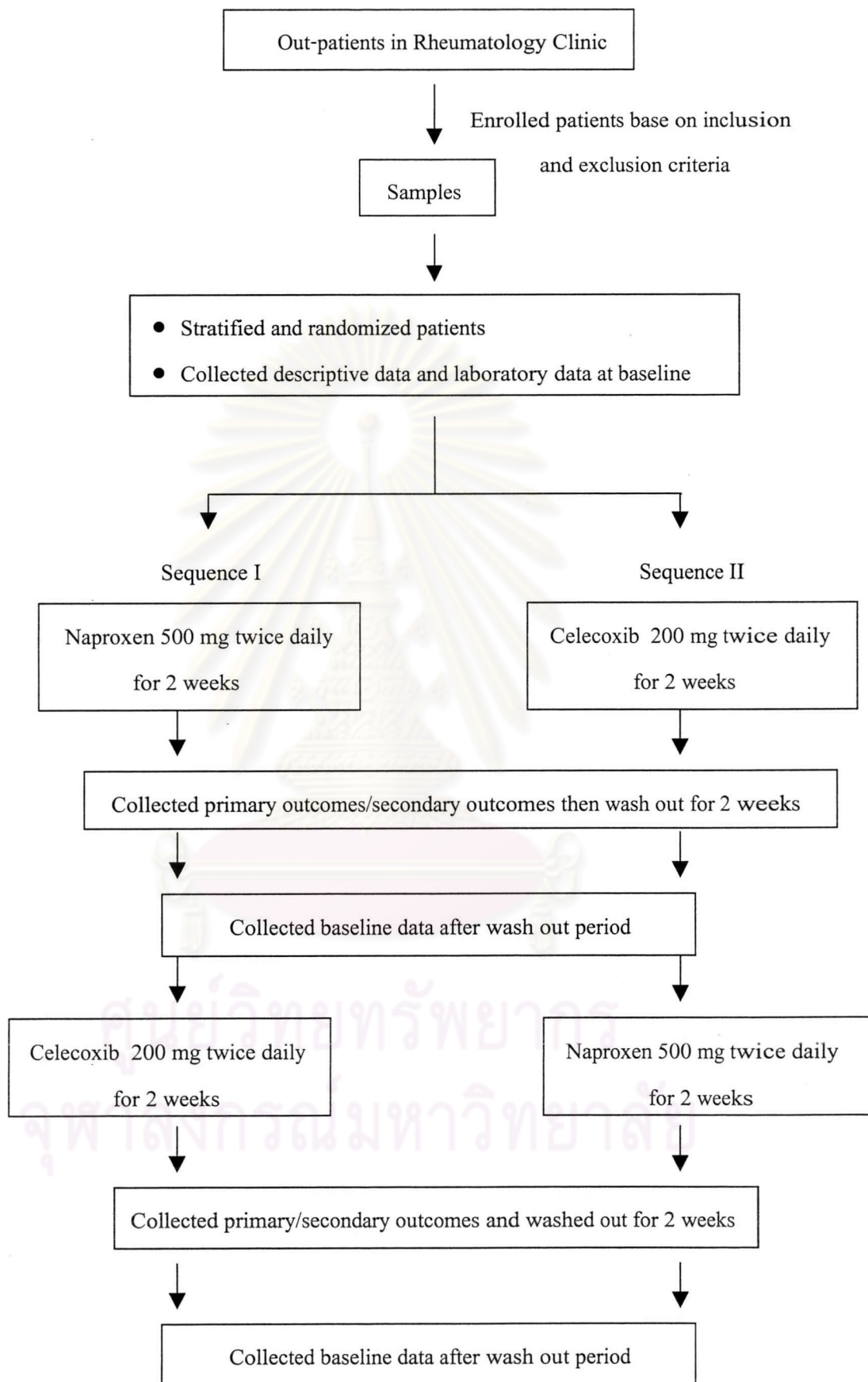9. Analyze, discussion and conclusion the study

Figure 9: The study flow chart

Table 13: Overall schedule of patients in this study

Week 1	Visit no. 1	←		Wash out		
Week 2						→
Week 3	Visit no. 2	←		First treatment		
Week 4						→
Week 5	Visit no. 3	←		Wash out		
Week 6						→
Week 7	Visit no. 4	←		Second treatment		
Week 8						→
Week 9	Visit no. 5	←		Wash out		
Week 10						→
Week 11	Visit no.6	←		Completed study	→	

Table 14: Summary of primary and secondary outcome collection

Visit No.	Demographic data	CBC	LFT	BUN	Scr	Na	K	BP	24-hour urine				Secondary outcomes
									Output	Cr	Na	K	
1	/							/					
2	/	/	/	/	/	/	/	/	/	/	/	/	
3	/	/	/	/	/	/	/	/	/	/	/	/	/
4	/	/	/	/	/	/	/	/	/	/	/	/	
5	/	/	/	/	/	/	/	/	/	/	/	/	/
6	/	/	/	/	/	/	/	/	/	/	/	/	

CBC = completed blood count

LFT = liver function test

BUN = blood urea nitrogen

Scr = serum creatinine

Na = sodium

K = potassium

BP = blood pressure

Cr = creatinine

6. Procedure of primary outcomes

1. Creatinine

Serum creatinine and urine creatinine were measured by Jaffe's reaction. Serum creatinine was measured at the last point of 24-hour urine collection.

2. Sodium and Potassium

Sodium and potassium in serum and urine were measured by Direct Ion Selective Electrode method.

3. Blood pressure measurement

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a mercury sphygmomanometer on the arm after fifteen minutes of rest in sitting position. Two consecutive blood pressure were measured apart five minutes (mean of two measurements was presented). Conditions that may affect blood pressure were separated by using the same material, observer and conditions (e.g., maintaining visit time, location and temperature). The heart rate was also monitored.

4. Edema

The measurement used four levels of Likert's scale (0 to +3) in accordance with body weight of patients that assessed by the same physician.

0 = pitting edema could not be seen with extensive pressure

+1 = patients occurred pitting edema when observed by extensive pressure at pre tibial area

+2 = patients occurred pitting edema when observed by normal pressure at pre tibial area

+3 = pitting edema could be seen obviously without any pressure

Criteria for significant edema has been at least one of described following

- Increased scale from baseline at least of 1 grade in edema in accordance with 2% weight gain

- Increased scale from baseline of more than or equal to 2 grades in accordance with or without weight gain

7. Data presentation and statistical analysis: analysis was conducted by using the data analysis software (SPSS for Windows version 10.07 and Number Cruncher Statistical System, NCSS 2002)

1. Descriptive statistic: proposed demographic data and baseline characteristic of all patients and subgroup of patients

2. Inferential statistic: proposed primary and secondary outcomes evaluation

   - Change of primary outcomes before and after receiving naproxen and celecoxib in each treatment group by pair T test analysis. Significant level was set at p value < 0.05

   - Compared primary outcomes between celecoxib and naproxen from crossover design by crossover analysis. Significant level was set at p value < 0.05

   - Compared the occurrence of edema and secondary outcomes between celecoxib and naproxen by using Chi-Square test. Significant level was set at p value < 0.05