ผลของขนาดยาและระยะเวลาที่ได้รับยาอีนาลาพริล ต่อระดับฮีมาโตคริทในผู้ป่วยที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม

นางสาว ศรีสมร รัตนจินดา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรเภสัชศาสตรมหาบัณฑิต
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EFFECTS OF ENALAPRIL DOSAGES AND DURATION ON HEMATOCRIT LEVELS IN HEMODIALYSIS PATIENTS

Miss Srisamorn Rattanajinda

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By	Miss Srisamorn Rattanajinda
Field of study	Clinical Pharmacy
Thesis Advisor	Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.
Thesis Co-advisor	Boonthum Jirajan, M.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Pornpen Pramyotin..... Dean of Faculty of Phamaceutical Sciences

(Associate Professor Pornpen Pramyotin, Ph.D.)

THESIS COMMITTEE

.....Prapapuck Silapachote..... Chairman

(Associate Professor Prapapuck Silapachote, M.Sc. in Pharm.)

.....Duangchit Panomvana..... Thesis Advisor

(Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.)

.....Boonthum Jirajan..... Thesis Co-Advisor

(Mr. Boonthum Jirajan, M.D.)

.....Sookruetai Lekhyananda..... Member

(Ms. Sookruetai Lekhyananda, M.D.)

.....Pornanong Aramwit..... Member

(Assistant Professor Pornanong Aramwit, Ph.D.)

ศรีสมร รัตนจินดา: ผลของขนาดยาและระยะเวลาที่ได้รับยาอีนาลาพริลต่อระดับฮีมาโตคริทในผู้ป่วยที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม (EFFECTS OF ENALAPRIL DOSAGES AND DURATION ON HEMATOCRIT LEVELS IN HEMODIALYSIS PATIENTS) อาจารย์ที่ปรึกษา: รศ. ดร. ดวงจิต พนมวัน ณ อยุธยา, อาจารย์ที่ปรึกษาร่วม: น.พ. บุญธรรม จิระจันทร์: 117 หน้า. ISBN 974-17-3685-1

การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของยาอีนาลาพริล ผลของขนาดยาอีนาลาพริล และระยะเวลาที่ผู้ป่วยได้รับยาอีนาลาพริล ที่มีต่อระดับฮีมาโตคริท ในผู้ป่วยโรคไตวายเรื้อรังที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม วิธีวิจัยเป็นการวิจัยแบบย้อนหลังในผู้ป่วยที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม 57 ราย โดยแบ่งกลุ่มผู้ป่วยเป็น 2 กลุ่มย่อย กลุ่มย่อยที่ 1 ผู้ป่วยจำนวน 48 รายไม่เคยได้รับยาอีนาลาพริลมาก่อน แล้วได้รับยาอีนาลาพริล เป็นเวลานานอย่างน้อย 4 เดือน ขณะที่กลุ่มย่อยที่ 2 จำนวน 9 ราย เคยได้รับยาอีนาลาพริลมาก่อนไม่น้อยกว่า 4 เดือน แล้วหยุดยาอีนาลาพริล เป็นเวลานาน 4 เดือน เก็บข้อมูลระดับฮีมาโตคริทของผู้ป่วย เมื่อผู้ป่วยได้รับยาอีนาลาพริลติดต่อกันเป็นเวลานาน 4, 8 และ 12 เดือน ผู้ป่วยที่คัดเลือกเข้าสู่การวิจัยที่ได้รับยาอีริโธรโพอีติน (รวมถึงผู้ป่วยที่ไม่ได้รับยาอีริโธรโพอีตินร่วมด้วย) จะต้องได้รับยาอีริโธรโพอีติน และยาอีนาลาพริลในขนาดคงที่ก่อนเข้าสู่การวิจัย และตลอดระยะเวลาที่ติดตามเก็บข้อมูล

ผลการวิจัยพบว่าผู้ป่วยกลุ่มย่อยที่ 1 มีระดับฮีมาโตคริทลดลงอย่างมีนัยสำคัญทางสถิติ (จาก 29.85±6.05% เป็น 26.79±5.99%, p<0.001) ในขณะที่ผู้ป่วยกลุ่มย่อยที่ 2 มีระดับฮีมาโตคริทเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ (จาก 29.11±5.11% ไปเป็น 31.44±5.59%, p=0.025) ร้อยละของระดับฮีมาโตคริทที่ลดลงเห็นได้ชัดเจนยิ่งขึ้นในผู้ป่วยที่ไม่ได้รับยาอีริโธรโพอีตินร่วมด้วย (-11.90±10.87% และ -7.19±7.43%, p=0.064) นอกจากนี้ยังพบว่าผู้ป่วยที่ได้รับอีนาลาพริลในขนาดเท่ากับหรือ สูงกว่า 10 มิลลิกรัมต่อวัน มีระดับฮีมาโตคริทลดลงมากกว่าผู้ป่วยที่ได้รับยาอีนาลาพริลในขนาดต่ำกว่า (p=0.090) มีผู้ป่วยเพียง 13 รายที่ได้รับอีนาลาพริล และอีริโธรโพอีตินในขนาดคงที่ตลอดระยะเวลานาน 12 เดือน ระดับฮีมาโตคริทของผู้ป่วยลดลงอย่างมีนัยสำคัญ เมื่อได้รับยาอีนาลาพริลเป็นเวลา 4 เดือนแรก (จาก 30.23±6.21% ไปเป็น 28.15±5.77%, p=0.001) จากนั้นระดับฮีมาโตคริทจะลดลงไปอีกเล็กน้อยใน 4 เดือนที่ 2 (เดือนที่ 8) พอถึง 4 เดือนที่ 3 (เดือนที่ 12) ระดับฮีมาโตคริทจะเริ่มคงที่หรือกลับเพิ่มขึ้นเล็กน้อย เพศ อายุ ระยะเวลาที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม ความถี่ที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม ตลอดจนระดับฮีมาโตคริทเริ่มต้นก่อนได้รับยาอีนาลาพริลไม่มีอิทธิพลต่อผลการลดระดับฮีมาโตคริทที่เกิดจากยาอีนาลาพริล

ยาอีนาลาพริล ขนาดยาอีนาลาพริล และระยะเวลาที่ได้รับยาอีนาลาพริล มีผลต่อระดับฮีมาโตคริทในผู้ป่วยโรคโรคไตวายเรื้อรังที่ได้รับการฟอกเลือดด้วยเครื่องไตเทียม โดยเฉพาะอย่างยิ่งในผู้ป่วยที่ไม่ได้รับยาอีริโธรโพอีตินร่วมด้วย

ภาควิชา.....เภสัชกรรม.....ลายมือชื่อนิสิต.....ศรีสมร รัตนจินดา.....

สาขาวิชา.....เภสัชกรรมคลินิก.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

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The effect of enalapril, enalapril dosages and duration of taking enalapril on hematocrit levels in hemodialysis patients were studied. The retrospective, before-after taking/stop taking enalapril crossover designs were carried in 57 hemodialysis patients. They were divided into two subgroups, 48 of them (subgroup I) were not taken enalapril and then taken enalapril for at least 4 months, while the rest of them (subgroup II) were taken enalapril at least 4 months and then stop taking enalapril for 4 months. Hematocrit levels were evaluated after patients had taken enalapril for 4, 8 and 12 months. The patients recruited and continue monitoring must on stable dose of erythropoietin (including those who did not receive erythropoietin) and stable dose of enalapril.

Hematocrit levels were significantly decreased from 29.85±6.05% to 26.79±5.99% ($p<0.001$) in subgroup I and were significantly increased from 29.11±5.11% to 31.44±5.59% ($p=0.025$) in subgroup II. The decrement in hematocrit levels was more prominent in the patients who were not treated with erythropoietin (-11.90±10.87% vs -7.19±7.43%, $p=0.064$). Higher dosage of enalapril up to 10 mg/day caused higher effect on the decrement in hematocrit levels ($p=0.090$). Only 13 patients were on the same dosage of enalapril and erythropoietin for 12 months, there was significant decrement in hematocrit levels at the first 4 months (from 30.23±6.21% to 28.15±5.77%, $p=0.001$), the decrement was slightly further at the second 4 months (8 months), then was stable or slightly increased at the third 4 months (12 months). Sex, age, duration of hemodialysis, frequency of hemodialysis and baseline hematocrit levels did not show significant influence on the effect of enalapril on hematocrit levels.

Enalapril, enalapril dosages and duration of taking enalapril affected hematocrit levels in hemodialysis patients, especially the patients who were not treated with erythropoietin.

Department.....Pharmacy.....Student's signature.....Srisamorn Rattanajinda

Field of study.....Clinical pharmacy.....Advisor's signature.....Duangchit Panomvana

Academic year.....2005.....Co-advisor's signature.....Boonthum Jirajan

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LIST OF ABBREVIATIONS

ACE	angiotensin-converting enzyme
ACEI	angiotensin-converting enzyme inhibitor
Ac-SDKP	N-acetyl-seryl-aspartyl-lysyl-proline
ALT	alanine aminotransferase
AMI	acute myocardial infarcttion
APTT	activated partial thromboplastin time
ARB	angiotensin II blockers
AST	aspartate aminotransferase
AT II	angiotensin II
AUC	area under the curve
AV	arteriovenous
BFU-E	burst-forming units-erythroid
BUN	blood urea nitrogen
CFU-E	colony-forming units-erythroid
CFU-GM	colony-forming units-granulocytic-monocytic
CFU-MK	colony-forming units-megakaryocytic
CHF	congestive heart failure
CKD	chronic kidney disease
CO	cardiac output
CNS	central nervous system
DM	diabetes mellitus
EPO	erythropoietin
ESR	erythrocyte sedimentation rate
ESRD	end-stage renal disease
G-6-PD	glucose-6-phophate-dehydrogenase

GFR	glomerular filtration rate
GI	gastrointestinal
HD	hemodialysis
HOT	hypertension optimal treatment
HTN	hypertension
Hct	hematocrit
IGF-1	insulin-like growth factor-1
IU	international unit
IV	intravenous
iPTH	intact parathyroid hormone
JNC-6	Joint National Committee on the Prevention and Treatment of Hypertension
K/DOQI	Kidney Disease Outcomes Quality Initiative Work Group
MAP	mean arterial blood presusure
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
PAN	polyacrylonitrile
PT	prothrombin time
RPGN	crecentric glomerulonephritis
rHuEpo	recombinant human erythropoietin
SD	standard deviation
SQ	subcutaneous
TIBC	total iron-binding capacity
TSAT	transferrin saturation
UA	urine analysis

CHAPTER I

INTRODUCTION

Anemia is a common problem in patients with kidney disease. Introduction of recombinant human erythropoietin (rHuEpo) into clinical practice has been a very important improvement for the management of anemia, a consistent clinical feature of end-stage renal disease (ESRD) [1]. However, the dose of erythropoietin required to correct anemia varies among the patients, and some can be unresponsive in spite of applying very high doses. The common causes for inadequate response to erythropoietin are iron deficiency, infection and inflammation, osteitis fibrosa, aluminium toxicity, folate or vitamin B_{12} deficiency, inadequate dialysis, and malnutrition. Nevertheless, the reason for incomplete response to erythropoietin is not completely clear, as some patients lacking theses factors can also be unresponsive to erythropoietin [2]. There were many studies investigated that angiotensin-converting enzyme inhibitors (ACE inhibitors) is the one factor that have the effect of erythropoietin responsiveness.

Enalapril maleate, a non-sulph-hydryl angiotensin-converting enzyme inhibitor, is a pro-drug whose pharmacological activity is dependent on its de-esterification in the liver to its active diacid metabolite, enalaprilat. Enalaprilat excretion occurs primarily *via* the urine and secondarily *via* the bile. The mean peak serum concentrations of enalapril and enalaprilat are greater and delayed in patients with chronic renal insufficiency. Steady-state enalaprilat serum levels are not achieved in hemodialysis (HD) patients because HD effectively reduces enalaprilat serum levels with a dialysis clearance of enalaprilat amounting to more than 50 ml/min. A strong link exists between enalapril serum levels and ACE inhibition, but not with the hemodynamic effects. Thus the optimum dosage of enalapril has not been determined.

However, 2.5 mg/day, 5 mg/day, or 5-40 mg/day of enalapril were successfully used to reduce blood pressure in HD patients without significant untoward events. ACE inhibitors are widely used in the treatment of hypertension, left ventricular dysfunction, diabetic nephropathy, and renal post-transplant erythrocytosis, and in preventing the progression of established renal disease of diverse causes [1].

In 1999, Macdougall IC described several possible mechanisms of ACE inhibitors that effects to the significant falling Hct level in patients stable on erythropoietin. (i) There is some evidence that angiotensin II can stimulate erythroid progenitor cell growth *in vitro*, and that captopril can inhibit this. (ii) A natural stem cell regulator called Ac-SDKP (N-acetyl-seryl-aspartyl-lysyl-proline) has been identified recently which inhibits the recruitment of pluripotent haemopoietic stem cells and normal early progenitors into the S-phase. ACE inhibitors have been found to increase plasma levels of Ac-SDKP markedly, and hence inhibit erythroid growth. (iii) A recent study by Morrone *et al*. has also suggested that insulin-like growth factor-1 (IGF-1) plays a role in the ACE inhibitor related decrease in Hct in patients with transplant polycythaemia. Serum erythropoietin and IGF-1 levels were significantly higher in patients with this condition than in a control group without transplant polycythaemia. ACE inhibitors significantly reduced Hct, IGF-1 and erythropoietin levels, and a direct relationship was found between Hct and serum IGF-1 levels, but not between Hct and serum erythropoietin levels. (iv) Similarly, ACE inhibitors have been shown to reduce production of interleukin-12, a cytokine known to stimulate erythropoiesis. Thus, it is likely that there are several means by which ACE inhibitors could inhibit erythropoietic activity [3].

A number of studies have reported ACE inhibitors antagonized the effects of erythropoietin on the treatment of anemia in HD patients. In 1986, Hirakata *et al.* evaluated the effect of captopril administration average dose 19.3 mg/day, ranging from 9 to 34 mg in 48 days, ranging from 23 to 68 day in 13 HD patients, they found that angiotensin II was significantly reduced by captopril, accompanied with decreased

reticulocytosis and reduce erythropoietin production leading to the worsening of anemia in patients [4]. In 1995, Dhondt *et al*. searched for cross-sectional study in 49 HD patients who received erythropoietin alone (n=27) and received erythropoietin with ACE inhibitors (n=22; 16:lisinopril 2.5-20 mg/day, 4:captopril 12.5-37.5 mg/day, 1:perindopril 4 mg/day and 1:ramipril 2.5 mg/day) for 3 months, the study demonstrated that more exogenous erythropoietin was required in ACE inhibitors treated chronic HD patients to achieve the same mean Hct of 33% [5]. After that in 1997, Matsumura *et al*. examined retrospective study in 108 HD patients who received erythropoietin alone (n=59) and received erythropoietin with ACE inhibitors (n=49; 31:enalapril, 10:imidapril, 2:captopril, 2:cilazapril, 2:benazepril and 2:temocapril), the data suggested that ACE inhibitors administration increased erythropoietin maintenance doses in HD patients [6]. In addition, in 1998, Albitar *et al.* evaluated from a prospective, non-randomized, controlled trial by follow up 1 year in 60 erythropoietin treated HD patients who taken enalapril (n=20, 10 and 20 mg/day), nifedipine (n=20, 40 mg/day), and control group (erythropoietin alone; n=20), they concluded that high-dose enalapril increased erythropoietin requirement [1] similarly to the observation by other previous studies [7], [8], [9].

On the other hand, some studies reported that ACE inhibitors therapy did not appear to affect response to erythropoietin in chronic dialysis patients. In 1998, Charytan *et al.* using a retrospective design studied in 175 erythropoietin treated HD patients (143 erythropoietin alone, 32 erythropoietin with enalapril 11$\pm$10.7 mg/d; range 2.5-40 mg/day; 50% of these patients taken enalapril <10 mg/day) for 7 months, they summarized that the dose or duration of ACE inhibitor therapy did not affect hemoglobin and hematocrit, thus, ACE inhibitors therapy did not appear to affect response to erythropoietin in chronic dialysis patients [10]. Likely to the study in 2000, Albu Alfa *et al.* evaluated for cross over prospective study in 33 erythropoietin treated HD patients for 4 months, the study reported that no significant increase in erythropoietin requirements in HD patients administered an ACE inhibitor compared

with other antihypertensive medications [11]. In addition, a recent study in 2001, Hayashi *et al.* using retrospective design studied in 2,213 erythropoietin treated HD patients (329 erythropoietin with ACE inhibitors which mostly were taken enalapril 5 mg/day and captopril 37.5 mg/day, 1,884 erythropoietin alone) for 3 months, they found that no statistically significant differences between the two groups, even if they use a simple comparison (p=0.941), a multiple regression (p=0.308) and comparison between matched groups by propensity score (p=0.355) to analyse the data [12]. These were similarly to several previous studies [2], [13], [14].

Since previous studies reported controversial results which might be the causes of different doses of ACE inhibitors and erythropoietin in each study. Some administered high dose ACE inhibitors while some used low dose ACE inhibitors. Besides, they compared patients between different groups (patients with erythropoietin alone, patients with erythropoietin plus ACE inhibitors) which had different baseline Hct. Furthermore, previous studies did not evaluate and compare the effects of ACE inhibitors dosage on the erythropoietin dosage requirements. Although there was some study [4] about ACE inhibitors dosages but the subjects in each dosage group were too low to compare the effect.

Therefore, this retrospective study was designed to evaluate the effect of enalapril, enalapril dosages and enalapril duration on the Hct levels in HD patients using a before-after crossover design. The Hct levels of patients between before taking/stop taking enalapril and after taking/stop taking enalpril for 4, 8 and 12 months, will be compared based on the conditions of receiving stable dosages of erythropoietin and enalapril.

The purposes of this study

1. To study the effect of enalapril on hematocrit levels of hemodialysis patients between before taking/stop taking enalapril and after taking/stop taking enalapril for 4 months.
2. To study the effect of enalapril dosage on the percentage of decreasing in hematocrit levels before taking/stop taking enalapril and after taking/stop taking enalapril for 4 months.
3. To study the effect of duration of taking enalapril on the percentage of change in hematocrit levels when the patients had taken enalapril for 4, 8 and 12 months.

CHAPTER II

LITERATURE REVIEW

1. Hemodialysis

End-stage renal disease (ESRD) may result from primary injury to the nephron or develop secondary to systemic diseases such as diabetes mellitus, hypertension, and certain autoimmune disorders (e.g., systemic lupus erythematosus). Intrarenal diseases (e.g., polycystic kidney disease, primary glomerulonephritis), or postrenal factors (e.g., uretral obstruction and neoplasm) account for approximately 20% of cases. Exposure to toxic substances (e.g., amphotericin B, aminoglycosides, compound analgesics) is a rare cause of ESRD. As renal function declines to less than 10 to 15% of normal, accumulation of nitrogenous wastes and other toxins leads to uremia, which is clinically defined as symptomatic real failure. These uremic sign and symptoms encompass myriad complications affecting most major organ systems, including the cardiovascular, pulmonary, neuromuscular, and central nervous systems. If the patient does not receive a kidney transplant, dialysis become necessary to sustain life.

Hemodialysis is the effective dialysis that entails convective bulk fluid removal, ultrafiltration, and diffusion of toxic materials down a concentration gradient from blood to dialysate. Convection is the process by which solutes are lost during ultrafiltration. Ultrafiltration depends on the difference in blood and dialysate transmembrane colloid osmotic pressure, membrane permeability, and blood dilution. The use of high dialysate and blood flow rates with high-flux (larger-pore) and high-efficiency (larger surface area) dialyzers has necessitated precise ultrafiltration control systems to avoid excessive fluid loss, dehydration, and hypotension. [15]

2. Pharmacotherapeutic consideration for patients on dialysis

2.1 Anemia

The primary cause of anemia in patients with ESRD is a relative erythropoietin (EPO) deficiency, for which therapy with recombinant human EPO alfa (epoetin) has been available since the late 1980s. The kidneys synthesize about 90% of circulating EPO, and secretion increases in response to hypoxia. Although the hematocrit (Hct), an index of the red blood cell count, begins to decline when the serum creatinine is more than 2 mg/dl, it usually is maintained above 30% until GFR declines to 30 ml/minute or less. Others factors such as blood loss; iron, folic acid or vitamin B_{12} deficiency; severe renal bone disease; systemic infection or inflammatory illness; and aluminum toxicity may also contribute to the development of anemia in patients on dialysis. Because the cause of anemia in patients on dialysis often is multifactorial, multiple hematologic and iron studies should be assessed before the patient begins epoetin.

Fatigue, exertional dyspnea, dizziness, headache, angina, congestive heart failure, and decreased cognition are common in patients with ESRD. Reversal of the signs and symptoms of tissue oxygen deprivation and left ventricular hypertrophy, improvement in exercise capacity, and ultimately and improvement in the quality of life are the primary therapeutic goals of anemia management. Because the signs and symptoms of anemia tend to resolve in patients who have achieved and maintained Hct between 30 and 38%, the DOQI working group recommended a target Hct range of 33 to 36%.

Currently, epoetin is the therapy of choice for maintaining Hct levels in patients on dialysis. It is reasonable to begin epoetin therapy when the patient's Hct drops below 33%. Before epoetin therapy is initiated, iron status (serum ferritin and transferring saturation [TSAT]) should be assessed. To optimize hematopoiesis in patients on dialysis receiveing epoeitn, ferritin and TSAT values of at least 100 ng/ml and 20%, respectively, should be maintained.

Epoetin can be administered intravenously or subcutaneously at a dosage of 90 to 180 U/kg/week. Subcutaneous administration is recommended for all patients with ESRD. Although the bioavailability of subcutaneous epoetin is low, the half-life is prolonged and Hct response is at least as good as or better than with intravenous administration. The preponderance of data indicates that the target Hct may be maintained with weekly subcutaneous epoetin dosages that are about 30% lower than the intravenous dosages. [15]

2.2 Potential causes of poor response to erythropoietin therapy for anemia of chronic renal disease

1. Iron deficiency (relative or absolute)
2. Folate or vitamin B_{12} deficiency
3. Malnutrition
4. Inflammatory, infectious, or malignant process
5. Unrecoginzed hemoglobinopathy (e.g., thalassemias)
6. Severe osteitis fibrosa
7. Aluminum intoxication or elevated Al^{3-} stores
8. Severe uremia or inadequate dialysis
9. Unrecognized blood loss or hymolysis (nondialysis related)
10. Excessive known blood loss (dialysis related, vascular surgery)
11. Pharmacokinetic variability-consider switch of administration route (SQ $\leftrightarrow$IV)
12. Concurrent medication: marrow-suppressive agents; angiotensin converting enzyme inhibitors or possibly angiotensin receptor blockers [15]

3. Erythropoietin

3.1 Chemical structure and properties

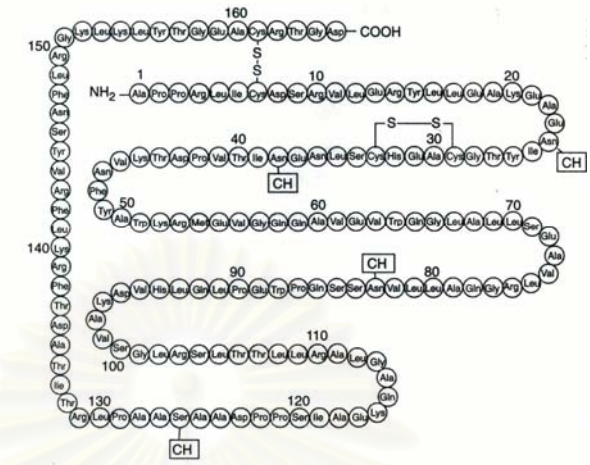


Figure 1: Chemical structure of erythropoietin [16]

Erythropoietin was the first human hematopoietic growth factor to be isolated. It was first purified from the urine of patients with severe anemia. Recombinant human erythropoietin (rHuEpo, epoetin alfa) is produced in a mammalian cell expression system using recombinant DNA technology. It is a heavily glycosylated peptide of 165 amino acids with a molecular weight of 30,400. It is measured in international units (IU). [17]

3.2 Pharmacology properties

Erythropoietin induces the production of erythrocytes (i.e., stimulates erythropoiesis) principally by stimulating the proliferation and differentiation of committed erythroid precursors (i.e., burst-forming units-erythroid [BFU-E], colony-forming units-erythroid [CFU-E]); CFU-E appear to be more sensitive and dependent on the effects of erythropoietin than BFU-E, responding in vitro to erythropoietin concentrations much lower than those required to stimulate BFU-E. Other marrow precursors, including colony-forming units (CFU)-megakaryocytic (CFU-MK), CFU-granulocytic-monocytic (CFU-GM), and pluripotent stem cells also may be increased with in vivo administration of erythropoietin. Stimulation of CFU-E and BFU-E

appears to be direct, while stimulation of CFU-MK and CFU-GM may occur as indirect feedback responses. Both erythroblasts and reticulocytes, the direct precursors (immature forms) of erythrocytes, are increased by erythropoietin as a result of its action on CFU-E and BFU-E. The hormone also stimulates the release of reticulocytes from bone marrow, and the synthesis of cellular hemoglobin is increased as a result of enhanced differentiation of CFU-E into erythroblasts.

In general, no changes in erythrocyte indices (e.g., mean corpuscular volume, erythrocyte width) are seen in patients receiving erythropoietin therapy, although increases in mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) have occurred in some patients. The effects of erythropoietin therapy in reversing shortened erythrocyte survival in uremic patients have been variable; limited evidence suggests that osmotic fragility of erythrocytes from hemodialysis patients decreases and erythrocyte half-life increases after erythropoietin therapy in predialysis patients, while other studies have demonstrated no such effects.

The usual serum concentration of endogenous erythropoietin in healthy individuals with normal hematocrit to 4-30 mU/ml; the erythropoietic response in patients with hypoxia, severe anemia from blood loss, or aplastic anemia may be associated with erythropoietin concentrations up to 1000 times greater than these levels. Most patients with anemia associated with chronic renal failure cannot mount this same erythropoeitic response and have an inappropriately low serum erythropoietin concentration for the degree of anemia present.

Erythropoietin appears to bind to two types of polypeptide receptors in erythroid progenitor cells-one high affinity and one low affinity- or possibly to different low- and high-affinity portions or chains of a receptor complex. Some data suggest that binding of the erythropoietin molecule to the receptor may stimulate nucleic acid synthesis and subsequent cell division in erythroid progenitor cells, although it also has been suggested that the hormone may prevent apoptosis

(programmed cell death) in such erythroid cells, allowing them to proceed with mitosis and terminal differentiation. Following receptor binding, erythropoietin apparently is internalized and metabolized by the target cell. Erythropoietin receptors are of the hormone-receptor variety but are not cross-reactive with any other hormone (e.g., insulin) or growth factor (e.g., colony-stimulating factors, various interleukins, transferring). Binding sites for erythropoietin have been identified in in vitro studies of isolated CFU-Es. Erythropoietin apparently does not bind to mature erythrocytes, granulocytes, monocytes, or plasma cells. In vitro studies have demonstrated receptors for erythropoietin in both human and animal placental tissue that may be similar to the erythroid cell receptor.

Plasma iron and ferritin concentrations decrease with new erythrocyte formation as a result of iron incorporation into these cells and subsequent mobilization of tissue iron stores. Intracellular free calcium concentrations have increased with addition of erythropoietin to bone marrow and cord blood erythroblast cultures; such increases in calcium may possibly initiate intracellular mechanisms for erythrocyte proliferation and differentiation. Increases in erythron transferring uptake parallel the increases in reticulocyte count and erythrocyte mass in both dialysis-dependent and predialysis patients who respond to erythropoietin therapy. [18]

3.3 Pharmacokinetic properties

3.3.1 Absorption

Because of its protein nature, epoetin alfa is destroyed in the GI tract and must be administered parenterally (e.g., via IV infusion, subcutaneous injection, intraperitoneal instillation). Systemic absorption of epoetin alfa is delayed and incomeplete following subcutaneous injection or intraperitoneal instillation. However, while serum concentrations peak sooner and are substantially higher with IV than subcutaneous injection of epoetin alfa, they are less sustained, and the IV route of administration generally offers no clinical advantage over the subcutaneous route

except in patients with existing accessible IV sites (e.g., hemodialysis patients). In fact, limited evidence suggests that subcutaneous injectin of epoetin alfa 3 times weekly can procuce a hemoglobin response similar to that with IV administration but a lower dosages; other evidence indicates that dosages of epoetin alfa required for maintenance therapy generally are lower with subcutaneous than IV injection. The decreased and variable systemic absorption of subcutaneously administered epoetin alfa relative to IV administration may result from the lipophilicity and/or relatively large size of the molecule; degradation by peptidases in the skin also may be responsible. With usual epoetin alfa dosages of 50-300 units/kg 3 times weekly given either subcutaneously or IV, detectable serum concentrations of erythropoietin are maintained for at least 24 hours.

Serum erythropoietin concentrations exhibit considerable interindividual variation with a given epoietin alfa dose and route of administration. There was shown in table 1.

Table 1: Peak serum erythropoietin concentrations [18]

Route of administration	Dose of administration (units/kg)	Peak serum erythropoietin concentrations (mU/mL)
Intravenous injection (in chronic renal failure)	80	1200-1800
	120	3200-4700
	150	3000-5000
Intravenous injection (in healthy adults)	150	3500
	300	7300
Subcutaneous injection	50	36
	150	144-226
	300	285-288

Peak serum erythropoietin concentrations are achieved within 4-24 hours following subcutaneous injection of usual therapeutic doses of epoetin alfa, and serum erythropoietin concentrations generally remain above baseline for 2-4 days. Following

IV doses of 50-300 units/kg of exogenous erythropoietin, serum erythropoietin concentrations generally decline to baseline levels within 1-3 days.

Peak serum drug concentrations following subcutaneous injection of epoetin alfa appear to be reduced (by up to 40-70% compared with the first-dose peak) with multiple-dose administration of the drug. Limited evidence suggests that bioavailability of the drug following subcutaneous administration into the thigh is increased compared with subcutaneous administration into the arm or abdomen.

3.3.2 Distribution

The distribution of epoetin alfa and the endogenous hormone in humans remains to be fully elucidated. Epoetin alfa appears to distribute into a single compartment with an apparent volume of distribution that approximates of slightly exceeds plasma volume (about 4-5% of body weight). Thus, extravascular distribution of the drug and endogenous hormone appears to be relatively minor. In both healthy individuals and dialysis patients, the volume of distribution has been estimated to be 21-80 mL/kg following a single IV dose, and 57-107 or 42-70 mL/kg at the start of multiple-dose therapy or after normalization of hematocrit (98-378 days), respectively; distribution characteristics of epoetin alfa are similar in predialysis patients. Little, if any, accumulation of the drug appears to occur at dosages of 50-150 units/kg given IV 3 times weekly. However, drug accumulation may occur following multiple subcutaneous doses because of prolonged absorption.

In contrast to apparent distribution characteristics in humans, extravascular distribution of epoetin alfa and the endogenous hormone appears to be substantially greater in animals. Epoetin alfa has been shown to distribute into the liver, kidney, spleen, lung, and bone marrow in animals. Desialylated epoietin alfa appears to undergo extensive extravascular distribution. In animals, 85, 9, 4 and 2% of extravascular desialylated drug reportedly distributes into the liver, kidney, lung, and spleen, respectively.

Results of studies in animals are equivocal regarding the potential for epoetin alfa to cross the placenta, although in vitro evidence suggests erythropoietin receptors are present in placental tissue of some animals. It is not known whether the drug distributes into milk in humans.

3.3.3 Elimination

The elimination characteristics of epoetin alfa and endogenous erythropoietin in humans remain to be fully established. Most currently available information on the elimination of the hormone comes from animal studies, and the relevance of these findings in humans is unclear. Serum concentrations appear to decline principally in a monoexponential (first-order) fashion, although biexponential elimination from serum has been described in animals and occasionally in humans, particularly at relatively low dosages. The elimination half-life of epoetin alfa following IV administration in healthy individuals and in patients with chronic renal failure ranges from 4-16 hours. There is some evidence that the elimination half-life of epoetin alfa may increase with increasing dosage, but a reduction in half-life during continuous dosing also has been reported. In patients with impaired renal function, a prolongation in elimination half-life relative to that in patients with normal renal function may occur; however, the elimination half-life does not appear to be affected by hemodialysis. The elimination characteristics of epetin alfa appear to be similar in patients undergoing hemodialysis or peritoneal dialysis.

With continuous dosing at IV doses of 15-500 units/kg administered up to 3 times weekly, the elimination half-life of epoetin alfa may decrease over time. In patients undergoing hemodialysis, mean elimination half-life was reduced by 20-40% after several weeks to months of such dosing; no further reductions generally were observed beyond 3-4 months of continued therapy. Similar reductions in half-life with multiple dosing have been observed in predialysis patients; the mean half-life of the drug decreased by 40% after 8 weeks of therapy in one study. Although it has been

suggested that this decrease in half-life may be related to increased clearance of the drug as more erythroid precursors are formed and increased numbers of erythropoietin receptors are made available, other studies have not found reductions in elimination half-life after continuous dosing.

The metabolic fate of endogenous erythropoietin and the recombinant hormone (i.e., epoetin alfa) is poorly understood. Although some in vitro and animal data suggest that the kidney may be involved in erythropoietin metabolism, limited evidence in humans suggests otherwise. It also has been suggested that the physicochemical characteristics of the glycoprotein would impede access to potential sites of renal metabolism, thus limiting any contribution of the kidney in the degradation of the endogenous and recombinant hormones. Current evidence from studies in animals suggests that hepatic metabolism contributes only minimally to elimination of the *intact* hormone, but desialylated epoetin alfa (i.e., terminal sialic acid groups removed) appears to undergo substantial hepatic clearance via metabolic pathways and/or binding. Desialylation and/or removal of the oligosaccharide side chains of erythropoietin appear to occur principally in the liver; bone marrow also may have a role in catabolism of the hormone. Elimination of desialylated drug by the kidneys, bone marrow, and spleen also may occur; results of animal studies suggest that proximal renal tubular secretion may be involved in renal elimination.

Approximately 10% or less of an administered dose of recombinant epoetin alfa is excreted unchanged in urine, which is similar to the elimination characteristics of the endogenous hormone. The effect of hepatic impairment on elimination of the hormone has not been elucidated.

Elimination of epoetin alfa is similar in patients with varying degrees of renal failure, suggesting predominantly nonrenal mechanisms of elimination. Total body clearance of epoetin alfa in patients with varying degrees of renal failure averages 0.09 mL/minute per kg (range: 0.06-0.12 mL/minute per kg).

Epoetin alfa apparently is not removed by hemodialysis; in fact, postdialysis serum concentrations of the hormone may exceed predialysis concentrations because of removal of excess extracellular fluid. Removal of the hormone from systemic circulation into peritoneal dialysate is small and contributes minimally to overall elimination of the drug. [18]

3.4 Dosage in anemia of Chronic Renal failure

Erythropoietin can be administered either subcutaneously (SQ) or intravenously (IV). Predialyssi and peritoneal dialysis patients typically receive erythropoietin SQ divided into 1 or 2 doses per week, whereas most hemodialysis patient are given erythropoeitin by the intravenous route, in a schedule that coincides with their hemodialysis sessions (i.e., three times weekly). The DOQI-recommended initial adult erythropoietin dosing range in terms of weekly total is 80 to 120 U/kg per week (SQ) divided into 2 or 3 doses or 120 to 180 U/kg per week (IV) divided into 3 doses. Younger pediatric patients (less than 5 years) may require 2 to 3 times these doses. The time to reach new steady-state hematocrit values after and erythropoietin dose adjustment ranges from 1 to 4 months and is influenced no only by the erythropoietin dose and iron stores, but also by the turnover rate of the existing red blood cell mass. At the aforementioned doses, the average rate at which hematocrit rises in usual responders is 1% per week (range 0.5 to 1.5%). A variety of dosing strategies are acceptable; however, most algorithms make erythropoietin dose adjustments as a fractional increment of the previous erythropoietin dose, based on the actual hematocrit value along with the rate of hematocrit rise (figure 2). Hematocrit monitoring is recommended at 1- to 2-week intervals, accompanied by erythropoietin dosage titration at approximately 2- to 4-week intervals until a stable hematocrit and erythropoietin dose have been achieved. For patients exhibiting an exaggerated or unusually rapid hematocrit rise (e.g., greater than 4% in a 2-week period or 8% in a 4-week period), erythropoietin dosage reduction is suggested,

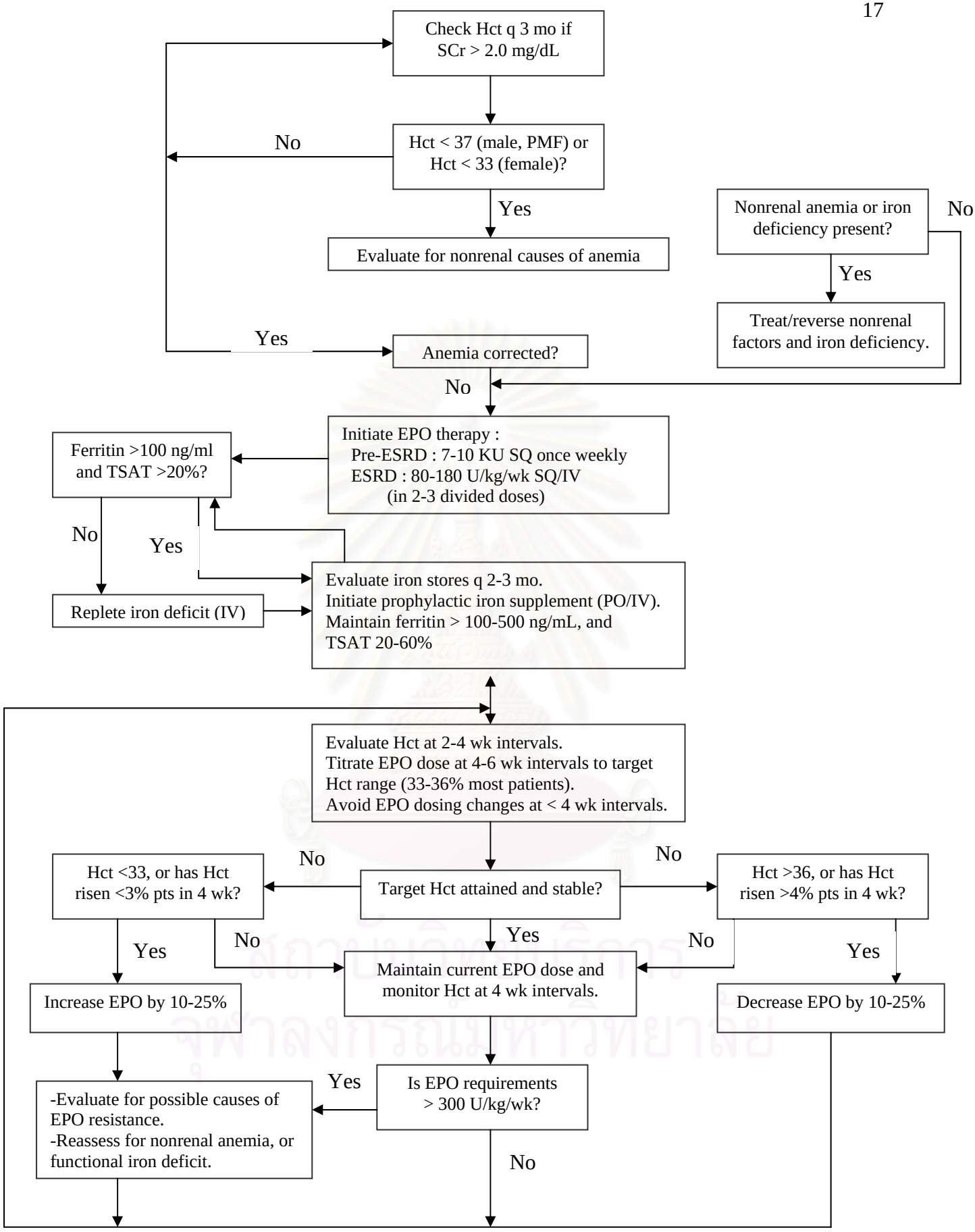


Figure 2: Algorithm for the management of anemia in Chronic Renal Disease. [19]

whereas suboptimal responders may benefit from early erythropoietin dosage increase to minimize the time to reach target hematocrit values (ideally within 2 to 4 months). Following stabilization of response, less frequent monitoring of hematocrit (every 2 to 4 weeks) and erythropoietin dose adjustments (every 4 weeks) generally suffice and tend to be more cost-efficient in centers managing large numbers of patients. Reticulocyte response is not routinely monitored in erythropoietin recipients demonstrating an adequate response. [19]

3.5 Adverse effects

3.5.1 Hypertension

The most frequent adverse effect observed in patients receiving epoetin alfa for anemia of chronic renal failure is development or exacerbation of hypertension. The risk of hypertensive episodes is greatest in patients with chronic renal failure who have preexisting hypertension or a history of hypertensive disease; however, up to 80% of patients with chronic renal failure have a history of hypertension. Patients with extremely low baseline hematocrits (e.g., less than 22%) also may be at risk for the development of hypertension. The risk of hypertensive episodes appears to be low to nonexistent in patients with normal renal function compared with that in patients receiving the drug for anemia of chronic renal failure; similar hypertensive episodes have been reported in patients with chronic renal failure following red blood cell transfusions. Although a direct causal relationship to the drug has not been established, hypertensive encephalopathy with or without subsequent seizures of the tonic-clonic type has been reported with increases in blood pressure in chronic renal failure patients receiving epoetin alfa, and correction of anemia should be performed slowly to minimize the risk of such complications.

The etiology of the hypertension reported in patients receiving epoetin alfa therapy is unclear; however, it usually is noted within the first 3 months of therapy and may be related to the rate or extent of increase in hematocrit. Hematocrit increases

exceeding 4% in a 2-week period reportedly may be associated with an increased risk of adverse hypertensive events in patients with chronic renal failure. A decrease in compensatory vasodilation of the vascular system associated with increases in hematocrit and correction of anemia, either by transfusion or epoetin alfa therapy, may result in an increase in total peripheral resistance. An increase in the viscosity of whole blood, but not plasma, with increases in hematocrit and/or erythrocyte count and possibly platelet count, also has been implicated in the increase in total peripheral resistance. Limited in vitro evidence suggests that increased circulation hemoglobin binds to or chemically inactivates a vascular endothelium-derived relaxant factor (possibly nitric oxide), which blocks the vasodilatory effects of this factor in vivo and produces a rebound vasoconstriction; however, the clinical importance, if any, of this effect has not been established. Most evidence suggests that epoetin alfa does not possess direct vasopressor effects.

Limited evidence suggests that cautious adjustment of dialysis or “dry” weight (e.g., weight after correction of clinical volume overload and optimization of sitting blood pressure without inducing orthostatic hypotension) may result in decreased plasma volume and prevention or control of adverse hypertensive effects of epoetin alfa in patients with chronic renal failure. However, such adjustments should be performed carefully to prevent hypovolemia and preferably, prior to initiating epoetin alfa therapy.

3.5.2 Hematologic effects

An increased incidence of partial or complete clotting at the site of vascular access (arteriovenous [AV] fistula) has been observed in renal dialysis patients receiving epoetin alfa. A direct correlation between an epoetin alfa-induced increase in hematocrit and the rate of thrombotic events has not been shown, and little to no change in activated partial thromboplastin time (APTT) and prothrombin time (PT) or fibrinogen concentration has been reported in patients or healthy individuals receiving

the drug. However, thrombotic complications have been attributed to epoetin alfa-induced increases in erythrocyte count and whole blood viscostity in up to 13% of patients undergoing hemodialysis. In one patient whose AV fistula had been functional for 10 years, AV thrombosis occurred following an increase in hematocrit from 18% to 40% with epoetin alfa therapy. Vascular access clotting has been reported to occur at a rate of 0.25-0.3 events per patient-year of epoetin alfa therapy, and heparin anticoagulation requirements during dialysis may be increased in patients receiving epoetin alfa. While most patients require only modest increases in heparin dosage to prevent clotting complication, dosage increases of up to twofold reportedly have been required in some patients.

3.5.3 Nervous system effects

Seizures (tonic-clonic [grand mal]) have been reported occasionally in patients receiving epoetin alfa. In clinical trials, up to 5% of dialysis patients treated with epoetin alfa experienced seizures, more frequently within the first 3 months of therapy. In most cases, seizures have been attributed to a precipitous rise in blood pressure associated with overly rapid correction of hematocrit; an associated hypertensive encephalopathy also has been described. Similar seizure activity has been observed in patients with chronic renal failure receiving transfusions for correction of hematocrit. In some cases, seizure activity may be preceded by sever headache and increased blood pressure, and may occur as single episodes of the tonic-clonic type not requiring specific treatment. If seizures develop during epoetin alfa therapy, the etiology of the seizure should be determined and the drug discontinued if hematocrit is elevated and hypertension is present. Therapy with epoetin alfa generally has been resumed in such patients without recurrence of seizure activity; however, discontinuance of the drug has been required in some cases.

3.5.4 Renal and electrolyte effects

Predialysis increases in serum concentrations of potassium, blood urea nitrogen (BUN), creatinine, uric acid, and phosphate have been reported with epoetin alfa therapy, especially in patients with chronic electrolyte abnormalities. It is unclear whether such increases are related to increased hemoglobin concentration, decreased efficiency of hemodialysis resulting from increased hematocrit, or lifestyle changes (e.g., increased appetite) brought about by the feeling of well-being associated with correction of anemia. A decrease in potassium and phosphate clearance in patients on hemodialysis has been associated with increasing hematocrit to greater than 35% with epoetin alfa therapy, while a decrease in dialyzer creatinine clearance has been associated with increasing hemoglobin concentration (e.g., from 7.5 g/dL to 10 g/dL). Most of these changes respond to dietary modification, administration of phosphate-binding antacids, adjustments in dialysis time or other dialysis parameters, use of a larger surface-area dialyzer, or adjusting the electrolyte concentrations of the dialysis fluid.

Severe, recurrent hyperkalemia has been reported infrequently with epoetin alfa and has been implicated as the cause of death in at least one patient. Hyperkalemia may be caused by increased dietary intake of potassium associated with epoetin alfa-induced improvement in appetite, decreased dialyzer efficiency, or release of potassium into plasma from the increased erythrocyte load during dialysis. Modifications in dialysis frequency and dialysis fluid and use of nutritional counseling may be required for the treatment and/or prevention of hyperkalemia in epoetin alfa-treated patients; administration of cationic exchange resins (e.g., sodium polystyrene sulfonate) also has been effective in acute and long-term treatment of hyperkalemia in such patients.

In patients experiencing hyperphosphatemia, and increase in calcium-phosphate product associated with joint pain, inflammation, swelling, and periarticular calcification has been observed.

3.5.5 Effects on dialyzer function

Although limited evidence suggests that decreases in hemodialyzer efficiency associated with increased hematocrit may be compensated for by adjusting dialyzer parameters (e.g., increasing high-efficiency hemodialysis prescription by 10-15% as the hematocrit approaches 40%), the circuit pressures and dialyzer function of high-flux or high-efficiency dialysis theorectically could be incompatible with the increased blood viscosity and hematocrit resulting from epoetin alfa therapy. Dialyzer fiber clotting and erythrocyete damage may occur as a result of interactions between increased blood viscosity and shear forces from the high flow rates used in these types of dialyzers; however, patients treated with epoetin alfa have undergone high-flux dialysis without serious complications when the hematocrit was maintained below 35%. Increased dialyzer fiber clotting also has been observed in patients not treated with epoetin alfa whose hematocrits exceeded 30%; slight increases in the heparin dosage administered during dialysis may be required to counteract this effect.

3.5.6 Flu-like syndrome

A flu-like syndrome has been reported rarely in patients receiving epoetin alfa, principally with IV infusion of the drug, but also with subcutaneous administration. This flu-like syndrome is characterized by the development of transient diaphoresis, chills, shivering, malaise, feeling of cold or warmth, myalgia, bone pain and arthralgia of the limbs and pelvis, generalized aches and pains (including chest, back, and/or flank pain), fever, paresthesias, and/or abdominal pain/cramps. The reaction reportedly appears within 90-120 miniutes of initiating the infusion and lasts for 2-12 hours. Although therapy with epoetin alfa has been discontinued in a few patients because of this adverse effect, the reaction generally is self-limiting and does not require dosage modification or preclude continued administration of the drug. To minimize the occurrence of this flu-like syndrome, some clinicians suggest administration of aspirin

or acetaminophen prior to injection of the drug and a reduction in the infusion rate during administration.

3.5.7 Other adverse effects

Nausea, vomiting, diarrhea, edema, arthralgias, and fatigue have been reported in more than 5% of chronic renal failure patients receiving epoetin alfa. Tachycardia, difficulty in maintaining ideal or “dry” postdialysis weight, cramps, nigh sweats, visual disturbances, exacerbation of acne, skin rash and urticaria (including petechial urticaria), petechial purpura, pruritus, transient local pain and/or stinging at the subcutaneous injection site, volume overload, shortness of breath, and conjunctival inflammation, redness, and/or injectin have been reported infrequently. Mixing at the time of administration epoetin alfa injection with bacteriostatic sodium chloride injection containing benzyl alcohol may ameliorate local discomfort associated with subcutaneous injection

Severe allergic reactions (manifestations not desbribed) reportedly have occurred rarely with epoetin alfa. An increase in unconjugated bilirubin (possibly associated with increased erythrocyte load and hemolysis) has been reported I some studies but not in others. [18]

4. Hypertension

Hypertension is a major modifiable cardiovascular risk factor in patients with ESRD and it is found in 60% and 24% of the hemodialysis and general U.S. populations, respectively. Inadequate hypertension control can accelerate atherosclerosis and increase cardiovascular morbidity secondary to heart disease and stroke. Predialysis mean arterial blood pressure (MAP) greater than 115 mm Hg has been associated with a significant increase in morbidity and mortality in patients on hemodialysis. A 10-mm Hg increase in MAP also is associated with a sevenfold increase in the risk of left ventricular hypertrophy and a twofold increase in the risk of heart failure. In patients with preexisting ischemic heart disease, mortality from myocardial infarction is the lowest when the treated diastolic blood pressure is reduced to 85 to 90 mm Hg. Recent results from the Hypertension Optimal Treatment (HOT) study indicate that the incidence of cardiovascular events is the lowest when diastolic blood pressure is reduced to 83 mm Hg or less. Moreover, subgroup analysis of patients with diabetes mellitus revealed a 51% lower incidence of major cardiovascular events in the target group (diastolic blood pressure 80 mm Hg or less) than in those who had a diastolic blood pressure of 80 to 90 mm Hg.

Despite the high prevalence of hypertension in the ESRD population, the mechanism of high blood pressure in these patients is not well understood. Sodium retention is believed to be one of the mechanisms responsible for the development of ESRD hypertension. However, extracellular volume expansion alone does not produce hypertension. In fact, patients on dialysis seldom have profound edema, and increased peripheral vascular resistance is found in almost all patients.

Hypertension in patients on dialysis can be treated by dialytic removal of sodium and water or the use of antihypertensive medications. Blood pressure patterns in these patients lack diurnal variation, and interdialytic blood pressure may be estimated by the predialysis and postdialysis blood pressure. In general, volume control by adequate dialysis is a more effective way to lower blood pressure than

pharmacologic therapy. In patients who have high blood pressure despite adequate fluid removal, use of antihypertensive medications is indicated. According to the recommendations from the sixth report of the Joint National Committee on the Prevention and Treatment of Hypertension (JNC-6), lifestyle modifications (e.g., reduced intake of dietary salt to less than 2 g/day, reduced saturated fat and cholesterol intake, smoking cessation, weight loss) are recommended as adjunctive therapy for all patients before pharmacologic therapy is initiated. Therapy selection should take into consideration other comorbid conditions such as diabetes mellitus, coronary heart disease, myocardial infarction, and depression. Lower starting dosages are necessary in older adults and when agents that are predominantly renally eliminated are being used. Higher dosages may be needed on nondialysis days than on dialysis days. Agents that are removable by dialysis may be given after dialysis on dialysis days. In general, ACE inhibitors, long-acting calcium channel blockers, β-blockers, and angiotensin receptor antagonists are effective for management of hypertension in patients with ESRD. The use of short-acting calcium channel blockers, especially in patients with diabetes mellitus, is controversial because of the potentially increased risk of cardiac events such as myocardial infarction and stroke. [20]

5. Angiotensin converting enzyme inhibitors

Renin, an enzyme produced by the kidney in response to a number of factors including adrenergic activity and sodium depletion, converts the circulating glycoprotein angiotensinogen into the biologically inert decapeptide angiotensin I, which is then changed by the angiotensin-converting enzyme (ACE or kininase II) into the highly potent vasoconstrictor angiotensin II (ATII). The angiotensin-converting enzyme is widely distributed, but its highest activity is in the endothelium of the pulmonary vasculature, probably because of the long length of pulmonary capillarieds. Other rennin-angiotensin systems are located in the brain, heart, and many other organs, the relevance of which, however, is uncertain. Bradykinin (an endogenous vasodilator

occurring in blood vessel walls) is also a substrate for ACE; it is probablya minor contributor to the vasodilator action of ACE inhibitors, except in patients without kidneys or other low-renin causes of hypertension. However, either bradykinin or one of the neurokinin substrates of ACE (such as substance P) may cause cough.

ATII acts on two G-protein-coupled receptors, of which the angiotensin AT_1 subtype accounts for all the classic actions of angiotensin. These include also stimulation of aldosterone (sodium-retaining hormone) production by the adrenal cortex.

When the ATII concentration in plasma is relatively high (i.e., approximately 100 pg/ml), the peptide causes direct arterial constriction. Inhibition of ATII formation reduces vasoconstriction, and blood pressure decreases. However, hypertensive patients with lower, or even normal, plasma concentrations of ATII also exhibit a depressor response to ACE inhibition. The mechanism of this effect is less clear. One possibility is that the ACE inhibitors act by blocking the tissue generation of ATII. ATII also can be produced by intrarenal rennin, in which case the peptide exerts an antinatriuretic and antidiuretic effect. Inhibition of intrarenal ATII formation by ACE inhibitors could lower blood pressure by promoting salt and water excretion in a manner similar to that of the diuretic agents. Finally, ACE inhibitors act by inhibiting brain ACE. An increase in intracerebral concentrations of ATII in experimental animals causes an elevation in arterial pressure mediated through activation of the sympathetic nervous system. The ACE inhibitors could reduce the activity of the sympathetic nervous system in a manner similar to that of centrally acting sympatholytic agents.

In addition, ATII stimulates cardiac and vascular smooth muscle cell growth, probably contributing to the progressive amplification of hypertension once the process is initiated. Limited studies to date have shown that the AT_2 receptor subtype is coupled to inhibition of muscle growth or proliferation.

ACE inhibitors reversibly inhibit angiotensin-converting enzyme that cleaves angiotensin I to form the potent vasoconstrictor ATII. These inhibitors also diminish the rate of bradykinin inactivation. They lower blood pressure by reducing peripheral vascular resistance without reflexly increasing cardiac output (CO), heart rate, or contractility. Vasodilatation occurs as a result of the combined effect of lower vasoconstriction caused by diminished levels of ATII and the potent vasodilating effect of increased bradykinin; renal blood flow may increase. By reducing circulating ATII levels, ACE inhibitors also decrease the secretion of aldosterone, resulting in decreased sodium and water retention. [20]

Table 2: Pharmacologic, pharmacokinetic, and dosing data of ACE inhibitors [20]

Drug	Pro drug	SH Group?	Protein binding (%)	Peak effect (h)	Metabo-lism	Elimina-tion	$T_{1/2}$ (h)	Daily dose	
								(Per day)	(mg)
Benazepril	Yes	No	95-97	2	Hepatic	Renal	10-11	1	10-20
Captopril	No	Yes	30	1-2	Hepatic (partially)	Renal	2-3	2-3	25-150
Cilazapril	Yes	No	Unknown	3-7	Hepatic + blood	Renal	3-9	1	1.5-5
Delapril	Yes	No	Unknown	1-2	Hepatic + blood	Renal	1-5	2	15-30
Enalapril	Yes	No	<50	2-4	Hepatic	Renal	6-11	1-2	5-40
Fosinopril	Yes	No	>90	2-4	Hepatic	Renal+ hepatic	>24	1	5-40
Lisinopril	No	No	None	4-8	None	Renal	12-13	1	5-40
Moexipril	Yes	No	Moderate	1-2	Hepatic	Renal	2-9	1	7.5-30
Perindopril	Yes	No	20	2-3	Hepatic	Renal	>24	1	2-8
Quinapril	Yes	No	95-97	1-2	Hepatic	Renal	2-3	1-2	5-40
Ramipril	Yes	No	50-60	3-6	Hepatic	Renal	14-30	1	2.5-10
Spirapril	Yes	No	89	4-6	Hepatic	Renal	>12	1	6
Trandolapril	Yes	No	80-94	4-6	Hepatic	Renal	>24	1	1-4

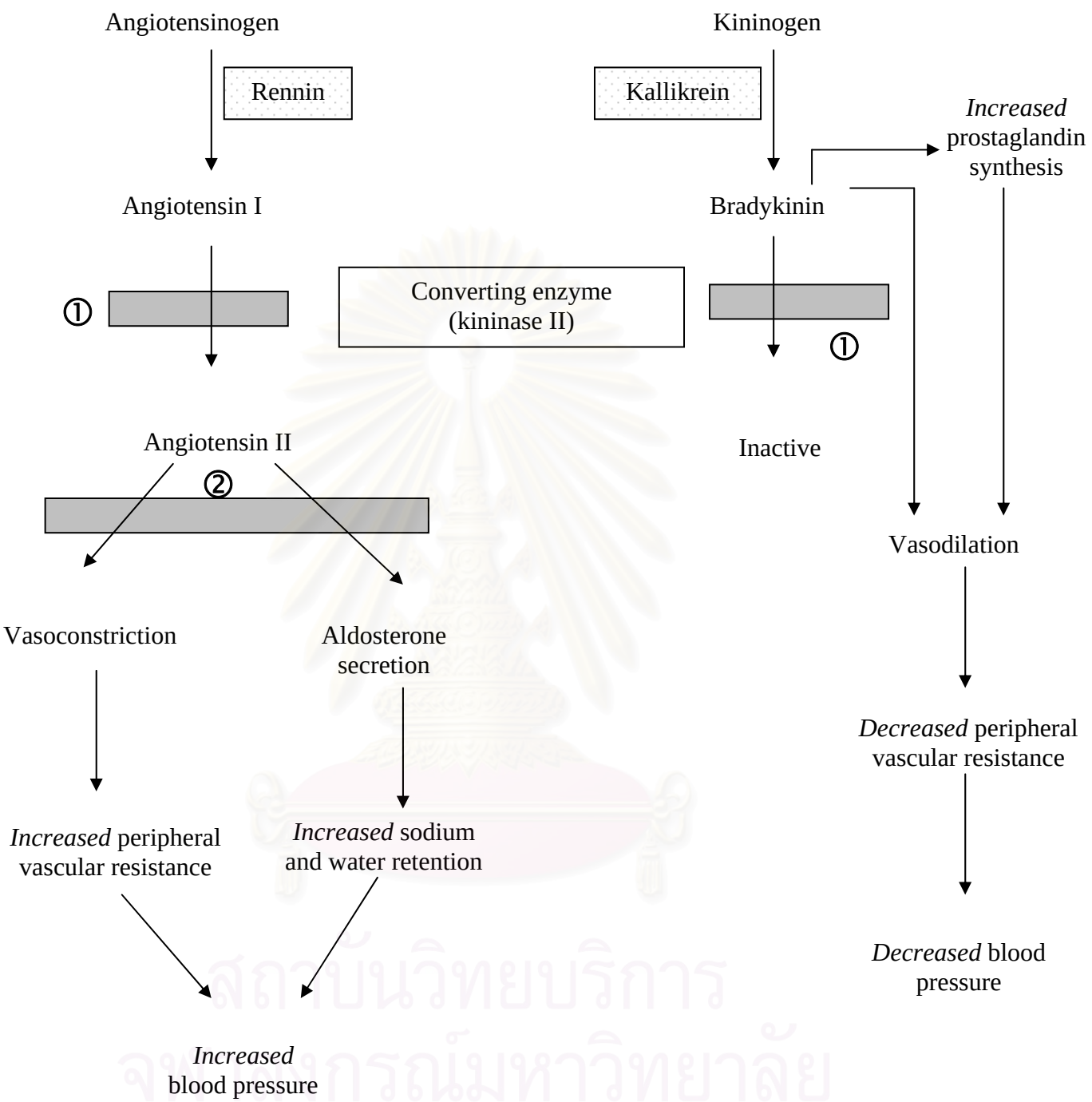
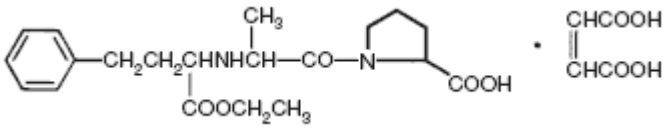


Figure 3: Sites of action of ACE inhibitors and receptor blockers. [20]

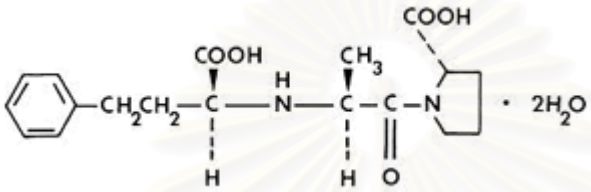
① Site of ACE blockade. ② Site of receptor blockade.

6. Enalapril and enalaprilat

6.1 Chemical structure and properties



Enalapril



Enalaprilat

Figure 4: Chemical structure of enalapril and enalaprilat [21]

Enalaprilat and enalapril are angiotensin-converting enzyme (ACE, bradykininase, kininase II) inhibitors. Enalapril, the ethylester of enalaprilat, is a prodrug and has little pharmacologic activity until hydrolyzed in the liver to enalaprilat. Enalapril is commercially available as the maleate salt and differs structurally from enalaprilat by the presence of an ethoxycarbonyl group rather than a carboxy group at position 1 of 1-alanyl-l-proline and by the presence of the maleate salt. These structural modifications result in increased GI absorption of enalapril compared with enalaprilat, which is poorly absorbed from the GI tract. Enalapril is structurally and pharmacologically similar to captorpil but contains a disubstituted nitrogen rather than a sulfhydryl group at positin 3 of 2-methyl-1=oxopropyl-l-proline. The lack of the sulfhydryl group in enalapril may result in decreased risk of certain adverse effects (e.g., cutaneous reactions, taste disturbances, proteinuria). [22]

6.2 Pharmacokinetic properties

6.2.1 Absorption

Enalapril maleate, unlike enalaprilat, is well absorbed following oral administration. Although enalaprilat is a more potent ACE inhibitor than enalapril, it is poorly absorbed from the GI tract because of its high polarity, with only about 3-12% of an orally administered dose being absorbed. Approximately 55-75% of an oral dose of enalpril maleate is rapidly absorbed from the GI tract in healthy individuals and hypertensive patients. Food does not appear to substantially affect the rate or extent of absorption of enalapril maleate. Following oral administration, enalapril maleate appears to undergo first-pass metabolism principally in the liver, being hydrolyzed to enalaprilat. Concomitant oral administration of enalapril maleate and hydrochlorothiazide has little, if any, effect on the bioavailability of either drug. Oral administration of the commercially available fixed combination containing the drugs reportedly is bioequivalent to concurrent administration of the drugs as individual preparations.

Peak serum enalapril concentrations of 40-80 ng/mL occur within about 0.5-1.5 hours following oral administration of a single 10-mg dose of enalapril maleate in healthy individuals or hypertensive patients. Peak serum enalaprilat concentrations reportedly increase proportionally with oral doses of enalapril maleate ranging from 2.5-, 5-, 10-, or 40-mg dose of enalapril maleate in these patients, average peak serum enalaprilat concentrations of 6-8, 15-28, 37-50, 70-80, or 123-150 ng/mL, respectively, occur within about 3-4.5 hours. Steady-state serum concentrations of enalaprilat were reached within 30-60 hours in patients with normal renal function receiving oral enalpril maleate dosages of 10 mg daily for 8 days; appreciable accumulation of the metabolite did not occur.

The hypotensive effect of a single oral dose of enalapril maleate is usually apparent within 1 hour and maximal in 4-8 hours. The hypotensive effect of usual

doses of the drug enerally persists for 12-24 hours but may diminish toward the end of the dosing interval in some patients. The reduction in blood pressure may be gradual, and several weeks of therapy may be required before the full effect is achieved. Following IV administration of enalaprilat, the hypotensive effect is usually apparent within 5-15 minutes with maximal effect occurring within 1-4 hours; the duration of hypotensive effect appears to be dose related, but with the recommended doses, the duration of action in most patients is approximately 6 hours. Plasma ACE inhibition and reduction in blood pressure appear to be correlated to a plasma enalaprilat concentration of 10 ng/mL, a concentration at which maximal blockade of plasma ACE is achieved. After withdrawal of enalapril or enalaprilat, blood pressure gradually returns to pretreatment levels; rebound hypertension following abrupt withdrawal of the drug has not been reported to date.

The onset and duration of hemodynamic effects of enalapril maleate appear to be slower and more prolonged than those of captopril. In patients with congestive heart failure, the hemodynamic effects of enalapril maleate are generally apparent within 2-4 hours and may persist for up to 24 hours after an oral dose.

6.2.2 Distribution

Distribution of enalapril into human body tissues and fluids has not been fully characterized. Approximately 50-60% of enalaprilat is bound to plasma proteins. Two binding sites have been identified, a low-affinity, high-capacity site and a high-affinity, low-capacity site. Drug bound to the latter site may represent enalaprilat bound to circulating serum ACE, possibly accounting for the prolonged terminal elimination of the drug.

Information on distribution into the CNS is limited, but enalapril appears to cross the blood-brain barrier poorly, if at all, and enalaprilat does not appear to distribute into the CNS. The drug did not accumulate in any tissue following multiple-dose administration in animals. The drug crosses the placenta. In a premature neonate

(35 weeks' gestation) whose mother received 20 mg of enalapril maleate daily for 17 days prior to delivery, plasma enalaprilat concentration soon after birth in the neonate was 28 ng/mL. Enalapril and enalaprilat are distributed into milk in trace amounts.

6.2.3 Elimination

Following oral administration, the half-life of unchanged enalapril appears to be less than 2 hours in healthy individuals and in patients with normal hepatic and renal functions, but may be increased in patients with congestive heart failure. Followeing oral administration of a single 5- or 10-mg dose of enalapril maleate in patients with congestive heart failure, the half-life of enalapril was 3.4 or 5.8 hours, respectively. Serum concentrations of enalaprilat, the active metabolite of enalapril, appear to decline in a multiphasic manner. Elimination of enalaprilat may also be prolonged in patients with congestive heart failure or impaired hepatic function compared with healthy individuals and patients with hypertension. Observations of serum concentrations of enalaprilat over long periods following oral or IV administration suggest that enalaprilat has an average terminal half-life of about 35-38 hours (range: 30-87 hours). The observed prolonged terminal phase may actually reflect enalaprilat binding to the high-affinity, low-capacity binding site of circulating serum ACE. The effective half-life for accumulation of enalaprilat (determined from urinary recovery) has been reported to average about 11 hours in healthy individuals with normal renal function.

Peak and trough enalaprilat concentrations and areas under the serum concentration-time curves (AUCs) may increase, time to peak and steady-state serum concentration may be delayed, and the effective half-life for accumulation may be prolonged in patients with impaired renal function. In patients with creatinine clearances less than 30 mL/minute, the effective half-life for accumulation of enalaprilat following multiple doses of enalapril maleate is prolonged. In patients with moderate renal impairment (i.e., creatinine clearances of 30-60 mL/minute), this half-

life is not substantially prolonged, and there appears to be a lack of correlation between AUCs and creatinine clearance. Decreased urinary excretion of enalapril may increase the extent of hydrolysis of enalapril to enalaprilat or may increase extrarenal elimination of the drug (e.g., via biliary excretion).

About 60% of an absorbed dose of enalapril is extensively hydrolyzed to enalaprilat, principally in the liver via esterases. About 20% appears to be hydrolyzed on first pass through the liver, this hydrolysis does not appear to occur in plasma in humans. Enalaprilat is a more potent ACE inhibitor than enalapril. There is no evidence of other metabolites of enalapril in humans, rats, or dogs. However, a despropyl metabolite of enalaprilat was identified in urine in rhesus monkeys, accounting for 13% of an oral dose of enalapril maleate. Hydrolysis of enalapril to enalaprilat may be delayed and/or impaired in patients with severe hepatic impairment, but the pharmacodynamic effects of the drug do not appear to be significantly altered.

Following oral administration, enalapril and enalaprilat are excreted in urine and feces. In healthy individuals, a mean of 60-78% (a mean of 43-56% as enalaprilat and the remainder as unchanged drug) of a 10-mg oral dose of enalapril maleate is excreted in urine within 24-48 hours. In a multiple-dose study (10 mg daily) in healthy individuals with normal renal function, urinary excretion of enalaprilat and total drug increased during the first 4 days of therapy and then stabilized; urinary excretion of the metabolite averaged 45% of the cumulative dose and that of total drug averaged 62%. It is not known whether enalapril and enalaprilat excreted in feces represent unabsorbed drug or that excreted via biliary elimination. Biliary excretion of enalapril and enalaprilat occurs in animals; however, this route of elimination has not been demonstrated in humans.

Renal clearance of enalaprilat and enalapril are reported to be approximately 100-158 and 300 mL/minute, respectively, in adults with normal renal function. The higher renal clearance of enalapril compared with that of the metabolite may indicate some degree of active tubular secretion of unchanged drug. Renal clearance may be

decreased in hypertensive patients. In geriatric individuals, renal clearance and/or volume of distribution may decrease.

Enalaprilat is removed by hemodialysis. The amount of drug removed during hemodialysis depends on several factors (e.g., type of coil used, dialysis flow rate); however, the hemodialysis clearance of enalaprilat is reportedly 62 mL/minute. Enalaprilat also appears to be removed by peritoneal dialysis. [21]

6.3 Dosage and administration

Use lower listed initial dose in patients with hyponatremia, hypovolemia, severe congestive heart failure, decrease renal function, or in those receiving diuretics.

Table 3: Dosage of enalapril and enalaprilat in children and adults [23]

Age / Route of administration	Disease	Dosage and administration
Children (1-16 years)	Hypertension	-initial 0.08 mg/kg (up to 5 mg) once daily -adjust dosage based on patient response -doses >0.58 mg/kg (40 mg) have not been evaluated in pediatric patients
	Congestive heart failure (investigational)	-initial 0.1 mg/kg/day increasing as needed over 3 weeks to 0.5 mg/kg/day have been used in infants
	Neonatal hypertension (investigational)	IV doses of enalaprilat -5-10 mcg/kg/dose administered every 8-24 hours have been used -monitor patients carefully, select patients may require higher doses

Table 3: Dosage of enalapril and enalaprilat in children and adults (cont.)

Age / Route of administration	Disease	Dosage and administration
Adults (oral : enalapril)	Hypertension	-2.5-5 mg/day then increase as require, usual therapeutic dose for hypertension : 10-40 mg/day in 1-3 divided doses -initiate with 2.5 mg if patient is taking a diuretic which cannot be discontinued. May add a diuretic if blood pressure cannot be controlled with enalapril alone.
	Congestive heart failure	As standard therapy alone or with diuretics, beta-blockers, and digoxin, initiate with 2.5 mg once or twice daily (usual range : 5-20 mg/day in 2 divided doses; target: 40 mg
	Asymptomatic left ventricular dysfunction	2.5 mg twice daily, titrate as tolerated to 20 mg/day
Adults (IV : enalaprilat)	Hypertension	-1.25 mg/dose, given over 5 minutes every 6 hours; doses as high as 5 mg/dose every 6 hours have been tolerated for up to 36 hours. -If patients are concomitantly receiving diuretic therapy, begin with 0.625 mg IV over 5 minutes -If the effect is not adequate after 1 hour, repeat the dose and administer 1.25 mg at 6-hour intervals thereafter; if adequate, administer 0.625 mg IV every 6 hours.
	Congestive heart failure	-Avoid IV administration in patients with unstable heart failure or those suffering acute myocardial infarction.
	Conversion from IV to oral therapy	-if not concurrently on diuretics : 5 mg once daily, subsequent titration as needed. -if concurrently receiving diuretics and responding to 0.625 mg IV every 6 hours, initiate with 2.5 mg/day.

6.3.1 Dosing adjustment in renal impairment

Table 4: Dosage of enalapril and enalaprilat in renal impairement patients [23]

Route of administration	Creatinine clearance	Dosage and administration
Oral enalapril	Clcr 30-80 mL/min	5 mg/day tlitrated upwares to maximum of 40 mg
	Clcr < 30 mL/min	2.5 mg/day titrated upward until blood pressure is controlled
	Heart failure patients	with sodium < 130 mEq/L or serum creatinine > 1.6 mg/dL initiate dosage with 2.5 mg/day, increasing to twice daily as needed increase further in increments of 2.5 mg/dose at >4-day intervals to a maximum daily dose of 40 mg
IV enalaprilat	Clcr >30 mL/min	Initiate with 1.25 every 6 hours and increase dose based on response
	Clcr <30 mL/min	Initiate with 0.625 mg every 6 hours and increase dose based on response
Hemodialysis	Moderately dialyzable (20-50%); administer dose postdialysis (e.g., 0.625 mg IV every 6 hours) or administer 20-25% supplemental dose following dialysis; Clearance: 62 mL/min.	
Peritoneal dialysis	Supplemental dose is not necessary, although some removal of drug occurs.	

6.3.2 Dosing adjustment in hepatic impairments

Hydrolysis of enalapril to enalaprilat may be delayed and/or impairment, but the pharmacodynamic effects of the drug do not appear to be significantly altered; no dosage adjustment. [23]

6.4 Adverse effects

6.4.1 Nervous system effects

Headache and dizziness occure in about 5% of patients receiving enalapril alone for hypertension, requiring discontinuance in 0.4 and 0.3% of patients, respectively, and occur in about 6 and 9%, respectively, of hypertensive patients receiving the drug in fixed combination with hydrochlorothiazide. In patients receiving enalapril for congestive heart failure, dizziness and headache occurred in approximately 8 and 2% of patients, respectively, and required discontinuance of the drug in 0.6 and 0.1%, respectively. Headache has been reported in about 3% of patients receiving enalaprilat. Fatigue has occurred in about 3% of patients receiving the drug alone for hypertension, requiring discontinuance in less than 0.1%, and has occurred in about 4% of hypertensive patients receiving the drug in fixed combination with hydrochlorothiazide. Fatigue, fever, and dizziness have been reported in 0.5-1% of patients receiving enalaprilat. Vertigo has occurred in about 2% of patients receiving enalapril for congesticve heart failure and required discontinuance in about 0.1% of patients. Insomnia, nervousness, peripheral neuropathy (e.g., paresthesia, dysesthesia, asthenia, and somnolence occur in about 0.5-2% of patients receiving enalapril alone or in fixed combination with hydrochlorothiazide. Hyperesthesia of the oral mucosa, CNS depression, malaise, nightmares, confusion, ataxia, and coldness of the extremities have been reported rarely.

6.4.2 GI effects

Diarrhea and nausea occur in about 1-2% of patients with hypertension receiving enalapril alone of in fixed combination with hydrochlorothiazide and in patients with congestive heart failure receiving the drug, and have required discontinuance of the drug in 0.2% or less of patients. Nausea has been reported in about 1% of patients receiving enalarpilat. Abdominal pain, vomiting, stomatitis, and

dyspepsia occur in 0.5-2% of patients receiving enalapril, and ulceration of the oral mucosa, ileus, melena, anorexia, glossitis, dry mouth, and flatulence have been reported rarely. Constipation has been reported in 0.5-1% of patients receiving enalaprilat.

6.4.3 Hepatic effects

A clinical syndrome that usually is manifested initially by cholestatic jaundice and may progress to fulminant hepatic necrosis (which occasionally may be fatal), has been reported rarely in patients receiving ACE inhibitors. The mechanism of this reaction is not known.

6.4.4 Cardiovascular effects

The most frequent adverse cardiovascular effect of enalapril or enalaprilat is hypotension (including postural hypotension and other orthostatic effects), which occurs in about 1-2% of patients with hypertension and in about 5-7% of those with congestive heart failure, following an initial dose or during extended therapy. Syncope occurred in approximately 0.5 or 2% of patients with hypertension or congestive heart failure, respectively. Hypotension or syncope has required discontinuance of therapy in about 0.1 or 2% of patients with hypertension or congestive heart failure, respectively, receiving enalapril. Enalapril-induced hypotension may occasionally be alleviated by dosage reduction, but severe hypotension has also occurred after low doses (i.e., a single 2.5- or 5-mg dose) of the drug. Orthostatic hypotension appears to occur more frequently during initiation of therapy and in patients with sodium depletion or hypovolemia. The risk of orthostatic hypotension associated with concomitant use of enalapril and a diuretic may be affected by the sequence of initiation of therapy with each drug; the risk may be higher when enalapril is added to diuretic therapy than when a diuretic is added to enalapril therapy.

When enalapril was used in fixed combination with hydrochlorothiazide in clinical trials in hypertensive patients, hypotension, orthostatic hypotension, and other orthostatic effects occurred in 0.9, 1.5, and 2.3% of patients, respectively. Syncope occurred in 1.3% of patients receiving the fixed combination, but the frequency of this effect can be minimized by proper titration of each drug separately and substitution with the combination preparation only when the optimum dosages correspond to the fixed ratio in the preparation.

Palpitation and chest pain occur in about 0.5-2% of patients with hypertension receiving enalapril alone or in fixed combination with hydrochlorothiazide. Tachycardia, bradycardia, and development or worsening of Raynaud's phenomenon have been reported rarely in patients receiving the drug. Cardiac arrest or cerebrovascular accident, possibly secondary to excessive hypotension in high-risk patients, pulmonary embolism and infarction, pulmonary edema, rhythm disturbances (including atrial tachycardia and bradycardia), flushing, and atrial fibrillation have been reported in about 0.5-1% of patients with hypertension or congestive heart failure. Angina or myocardial infarction was reported in about 1-1.5% of patients receiving enalapril for congestive heart failure in controlled and uncontrolled studies, and required discontinuance in about 0.1-3.0% of patients, but a similar incidence for these effects was reported in patients receiving placebo in controlled studies. Myocardial infarction was reported in 0.5-1% of patients receiving enalaprilat.

6.4.5 Renal effects

Deterioration in renal function, manifested as transient increases in BUN and serum creatinine concentrations, has occurred in about 20% of patients with renovascular hypertension, especially those with bilateral renal-artery stenosis or those with renal-artery stenosis in a solitary kidney. This effect was usually reversible following discontinuance of enalapril and/or diuretic therapy. Renal function should be monitored closely during the first few weeks of therapy in these patients. Transient

increases in BUN and serum creatinine concentrations have also occurred in about 0.2% of patients with hypertension, but without preexisting renal vascular disease, who were receiving enalapril alone. These effects occur more frequently in patients receiving concomitant diuretic therapy, in patients with congestive heart failure, and in patients with some degree of preexisting renal dysfunction. Dosage reduction of enalapril and/or dosage reduction or discontinuance of diuretic therapy may be necessary. The rapidity of onset and magnitude of enalapril-induced renal insufficiency in patients with congestive heart failure may depend in part on the degree of sodium depletion. Acute reversible renal failure, flank pain, oliguria, uremia, glycosuria, and proteinuria have been reported rarely in patients receiving enalapril. Urinary tract infection has been reported in about 1% of patients receiving enalapril for congestive heart failure in controlled and uncontrolled studies, but this effect occurred in about 2% of patients receiving placebo in controlled studies.

Because the rennin-angiotensin system appears to contribute substantially to maintenance of glomerular filtration in patients with congestive heart failure in whom renal perfusion is severely compromised, renal function may deteriorate markedly during therapy with an ACE inhibitor in these patients. Such drug-induced deterioration is generally well tolerated, and does not usually necessitate discontinuance of effective therapy with the drug when symptomatic improvement of the heart failure occurs. In addition, the magnitude of deterioration in renal function can usually be ameliorated by reducing the dosage of concomitantly administered diuretics and/or by liberalizing dietary sodium intake, since concomitant diuretic therapy and/or sodium restriction potentially increase the role of angiotensin II in maintaining glomerular filtration in these patients. In patients in whom renal perfusion pressure is very low and is further reduced by ACE-inhibitor therapy, however, deterioration in renal function may be clinically important. Patients with concomitant underlying diabetes mellitus may be at particular risk for developing renal insufficiency during ACE-inhibitor therapy. In some patients with severe congestive

heart failure, with or without associated renal insufficiency, treatment with an ACE inhibitor, including enalapril, may be associated with oliguria and/or progressive azotemia, and rarely with acute renal failure and/or death. The risk of developing function renal insufficiency appears to be higher during therapy with a long-acting ACE inhibitor such as enalapril than with a short-acting inhibitor.

6.4.6 Dermatologic and hypersensitivity reactions

The most frequent adverse dermatologic effect of enalapril is rash, which occurs in about 1.5% of patients and is usually maculopapular and rarely urticarial. Rash may sometimes be accompanied by pruritus, erythema, or eosinophilia, and has required discontinuance of the drug in approximately 0.3% of patients. Pruritis, without rash, and excessive sweating have been reported in 0.5-2% of patients receiving enalapril alone or in fixed combination with hydrochlorothiazide. Alopecia has been reported in 0.5-1% of patients receiving enalapril. A symptom complex, consisting of positive ANA titer, increased erythrocyte sedimentation rate (ESR), arthralgias and/or arthritis, myalgias, fever, serositis, vasculitis, leukocytosis, eosinophilia, photosensitivity, rash, and other dermatologic reactions has been reported in 0.5-1% of patients receiving enalapril therapy. Exfoliative dermatitis, toxic epidermal necrolysis, Stevens-Johnson syndrome, pemphigus, herpes zoster, and erythema multiforme have been reported rarely in patients receiving enalapril therapy.

Severe, sudden anaphylactoid reactions, which can be fatal, have been reported following initiation of hemodialysis that utilized a high-flux polyacrylonitrile [PAN] membrane (e.g., AN 69®) in patients receiving an ACE inhibitor. Manifestations of these reactions included nausea, abdominal cramps, burning angioedema, and shortness of breath; progression to severe hypotension can develop rapidly. Dialysis should be stopped immediately and aggressive supportive and symptomatic therapy should be initiated as indicated. Antihistamines do not appear to be effective in providing symptomatic relief. While it currently does not seem to be necessary to exclude the use

of ACE inhibitors in patients undergoing hemodialysis that involves PAN membranes, caution should be exercised during concomitant use. The mechanism of this interaction has not been established, and the incidence and risk of its occurrence remain to be elucidated. The possibility that ACE inhibitors may precipitate similar reactions in patients undergoing hemodialysis involving other membrane types (new or reprocessed) should be considered.

6.4.7 Hematologic effects

Decreases in hemoglobin and hematocrit averaging approximately 0.3 g/dL and 1%, respectively, occur frequently in hypertensive patients receiving enalapril alone or in fixed combination with hydrochlorothiazide, but rarely are clinically important unless another cause of anemia also exists. Enalapril-induced anemia has required discontinuance of therapy in less than 0.1% of patients. Hemolytic anemia, including cases of hemolysis in a few patients with glucose-6-phosphate-dehydrogenase (G-6-PD) deficiency, has been reported in patients receiving enalapril maleate therapy; a causal relationship has not been established.

Neutropenia (less than 1000 neutrophils/mm^3) and agranulocytosis, both associated with myeloid hypoplasia, have occurred rarely in patients receiving captopril. Several cases of neutropenia, agranulocytosis, or thrombocytopenia have been reported, and a causal relationship to enalapril cannot be excluded. Because of pharmacologic and structural similarities between captopril and enalapril and the current lack of sufficient data to establish the relative risk of these adverse hematologic effects in patients receiving enalapril, the possibility that bone marrow depression, neutropenia, and agranulocytosis could occur in patients receiving enalapril should be considered.

6.4.8 Effects on taste

Loss of taste perception and decrease in taste acuity have been reported infrequently during enalapril therapy. Hyperesthesia of the oral mucosa has occurred in at least one patient receiving enalapril but was reversible following discontinuance of the drug. Patients with intolerable captopril-induced taste disturbances may tolerate enalapril better.

6.4.9 Effects on potassium

Although small increases (i.e., by an average of 0.2 mEq/L) in serum potassium concentrations frequently occur in patients receiving enalapril without a thiazide diuretic, hyperkalemia (i.e., increases to greater than 5.7 mEq/L) occurs in approximately 1 or 4% of patients with hypertension or congestive heart failure, respectively, receiving the drug. In most cases, these were isolated increases that resolved despite continued therapy with the drug; however, hyperkalemia required discontinuance of enalapril therapy in about 0.3% of patients receiving the drug for hypertension. Hyperkalemia is less frequent in patients receiving enalapril and hydrochlorothizide concomitantly, occurring in about 0.1% of patients. Patients with diabetes mellitus, impaired renal function, or congestive heart failure and patients concomitantly receiving drugs that can increase serum potassium concentration (e.g., potassium-sparing diuretics, potassium supplements, potassium-containing salt substitutes) may be at increased risk of developing hyperkalemia during enalapril therapy; serum potassium concentration should be monitored frequently in these patients, and potassium intake should be controlled and therapy with drugs that can increase serum potassium modified or discontinued as necessary. The manufacturer recommends that potassium-sparing diuretics generally no be used in patients receiving enalapril for congestive heart failure.

6.4.10 Respiratory effects

Cough has been reported in 1.3 or 3.5% of patients receiving enalapril alone or in fixed combination with hydrochlorothiazide for hypertension, respectively, and in about 2% of those receiving the drug for congestive heart failure; discontinuance of the drug was required in less than 0.5% of patients. Nonproductive cough, particularly at night, may occur more frequently, especially in patients with chronic obstructive pulmonary disease. Some clinicians state that cough often is overlooked as apotential adverse effect of ACE inhibitors. The cough generally is persistent, is not associated with other respiratory symptoms, and is reversible following discontinuance of the drug. Nasal congestion also has been reported. It has been suggested that accumulation of kinins in the respiratory tract secondary to ACE inhibition may in part be responsible for cough and nasal congestion. If cough develops in a patient receiving enalapril, ACE inhibitor-induced cough should be considered as part of the differential diagnosis.

Dyspnea and wheezing, which may persist if therapy with the drug is continued, have been reported in about 1% or less of patients receiving enalapril. Pneumonia or bronchitis has been reported in about 1% of patients receiving enalapril for congestive heart failure. Asthma, upper respiratory infection, bronchospasm, pulmonary infiltrates, and rhinorrhea also have been reported in patients receiving enalapril maleate therapy. Angioedema has occurred in 0.2 or 0.6% of patients receiving enalapril alone or in fixed combination with hydrochlorthiazide, respectively, and if associated with laryngeal edema, may be fatal.

6.4.11 Other adverse effects

Muscle cramps, and impotence have been reported in 0.5-1% of patients receiving enalapril alone, and decreased libido has been reported rarely. These effects have occurred more frequently when the drug was administered in fixed combination with hydrochlorothiazide. Hearing loss, which was reversible following discontinuance of the drug, has been reported rarely; however, the mechanism of this adverse effect is not known. Pancreatitis, hepatitis or cholestatic jaundice, hepatic failure, sore throat, hoarseness, anosmia, conjunctivitis, dry eyes, tearing eyes, gynecomastia, and myalgia have been reported in patients receiving enalapril. Vulvovaginal pruritus, burning urination, and dysuria were reported in at least one patient receiving enalapril. Although a definite causal relationship to enalapril has not been established, elevations of serum hepatic enzymes and/or bilirubin concentrations have been reported rarely when enalapril was administered alone or in fixed combination with hydrochlorothiazide. [21]

CHAPTER III

PATIENTS AND METHODS

1. Patients

Patients with chronic kidney disease from Kidney Foundation of Thailand who had been on maintenance hemodialysis for at least 1 year, were treated with enalapril at least for 4 months during July 1997 to June 2005 were screened into this study. Among these fifty-seven patients, twenty three of them were monitored for 8 months while thirteen of them were monitored for 12 months.

2. Study design

This study was a retrospective, before-after taking/stop taking enalapril crossover design.

3. Subjects

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 at least twenty years old.
2. He/she had chronic kidney disease and received hemodialysis at the Kidney Foundation of Thailand.
3. He/she was on maintenance hemodialysis for at least 1 year.

4. He/she was dialysed two to three times a week.
5. He/she who was treated with enalapril before recruiting to the study, had to take stable dosage of enalapril for at least 4 months.
6. He/she who received erythropoietin before recruiting to the study, had to receive stable dose of erythropoietin at least 4 months before taking/stop taking enalapril and 4 months after taking/stop taking enalapril, as shown in figure 5.1.

Exclusion criteria

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

1. He/she was allergy to enalapril.
2. He/she had allergy history to ACE inhibitors or angiotensin II receptor blockers (ARBs).
3. He/she was required to be treated with packed red cell or parenteral iron supplement within 4 months before taking/stop taking enalapril and 4 months after taking/stop taking enalapril.
4. He/she was required to give or changed the dosage of erythropoietin within 4 months before taking/stop taking enalapril and 4 months after taking/stop taking enalapril.
5. He/she was required to stop or change the dosage of enalapril within 4 months before taking/stop taking enalapril and 4 months after taking/stop taking enalapril.
6. He/she was admitted into the hospital for more than 7 days.

4. Sample size

The sample size of this study was calculated from this formula

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

A sample-size determination was based on data obtained from a retrospective study; the special problem report (The effect of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II blockers (ARBs) to erythropoietin dose in hemodialysis patients at Rajavithi Hospital).[24]

Where N = number of sample size

Sp = standard deviation of hematocrit level

S1 = the patients who were received erythropoietin; n=8 S1=2.71

S2 = the patients who were received erythropoietin and ACEI/ARB; n=6, S2=1.15

$$Sp^2 = \frac{[n_1 - 1]S_1^2 + [n_2 - 1]S_2^2}{n_1 + n_2 - 2} = \frac{[8-1]2.71^2 + [6-1]1.15^2}{8+6-2} = 4.84\%$$

Z_α at 95% CI ; Z_α = 1.96

Power of 80% ; Z_β = 0.84

Clinical difference; D = 4 means that if the defference in hematocrit levels before taking/stop taking enalapril and after taking/stop taking enalapril for 4 months were more than or equal to 4% (percentage by volume of packed red blood cells (RBCs) in a whole blood sample), there were statistically significant different.

The sample size was then calculated to be:

$$N / group = \frac{[1.96 + 0.84]^2 \, 4.84^2}{4^2} = 12$$

After a 20% dropout augmentation for potential dropouts, a sample size of 15 is needed to detect the predefined difference of 4% using a 95% two-tail and 80% one-tail significance for α and β errors, respectively.

5. Steps of the study

1. The protocol of this study had to be approved by the Ethic Committee of Rajavithi Hospital.
2. Investigator prepared all the materials required in this study (instrument and record forms).
3. The patients were recruited into the study based on the inclusion/exclusion criterias.
4. The demographic data of patients who met the inclusion criterias were collected; gender, age, dry weight, height, duration of hemodialysis, frequency of dialysis, causes of chronic kidney disease, and comorbid conditions.
5. Laboratory data of patients before taking/stop enalpril and after taking/stop enalapril for 4 months were recruited; blood urea nitrogen (BUN), serum creatinine, uric acid, serum sodium, serum potassium, serum chloride, serum bicarbonate, serum calcium, serum phosphate, serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), intact parathyroid hormone (iPTH), serum ferritin, total iron-binding capacity (TIBC), and serum iron.
6. The initiation of the study was the time when the patients start or stop taking enalapril.

7. The enrolled patients were categorized into two groups;
   7.1. The patients who had not taken enalapril at least 4 months before and then start taking enalapril (Subgroup I; A01-A48) for 4 months (and/or for 8 and/or 12 months).
   7.2. The patients who had taken enalapril at least 4 months before and then stop taking enalpril (Subgroup II; B01-B09) for 4 months.

   If the patients had taken enalapril before, then, stop taking enalapril and start taking enalapril again, the patients should stop taking enalapril for at least 4 months (wash out period) before start taking enalapril again. These patients were classified into the same group as 7.1.
8. Monitored and recorded the following data:
   8.1. The dosages of enalapril and erythropoietin that the patients were received.
   8.2. Hematocrit levels of patients at time before taking/stop taking enalapril and after taking/stop taking enalapril for 4 months. For patients who were on stable dosages of enalapril and erythropoietin for 8 months or 12 months, the hematocrit levels were also recorded at the 4^{th} week of the 8^{th} month and the 12^{th} month of the patients who were monitored for 8 and 12 months respectively.

6. Statistical analysis

Analysis was conducted by using the data analysis software (SPSS for window version 11.5).

1. Descriptive statistics
   1.1. Demographic data of all patients were presented as descriptive statistic (mean±SD).

2. Inferential statistics

2.1. Laboratory data of all patients before taking/stop taking enalapril and after taking/stop taking enalapril for 4 months were compared by using Paired T-test (most of the time, significant levels were set at p-value <0.05).

2.2. Hematocrit levels before taking/stop taking enalapril and after taking/stop taking enalapril for 4, 8 and 12 months were compared by using Paired T-test (most of the time, significant levels were set at p-value <0.05).

2.3. The percentage of changes of Hematocrit levels in patients who were taking enalapril in different doses (≤5, 10, 20 and 40 mg/day) were compared by using independent T-test and ANOVA analysis, significant levels were normally set at p-value <0.05.

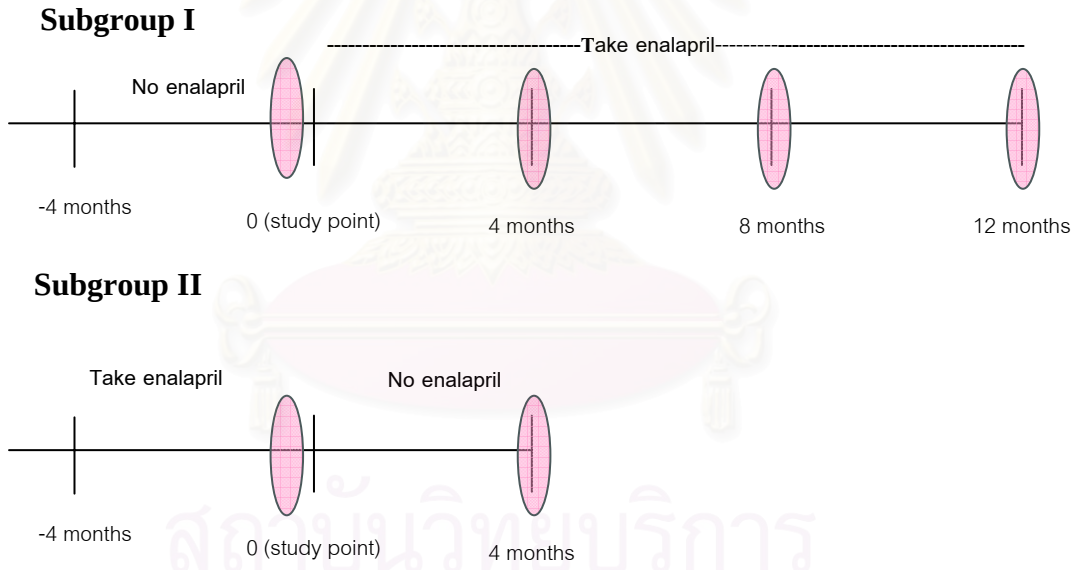


Figure 5.1: Flow chart showing the study point (time 0) of the two subgroups.

-Subgroup I means the patients who did not take enalapril at least 4 months before the study and then took enalapril for 4, 8 and 12 months.

-Subgroup II means the patients who took enalapril at least 4 months before the study and then stop taking enalapril for 4 months.

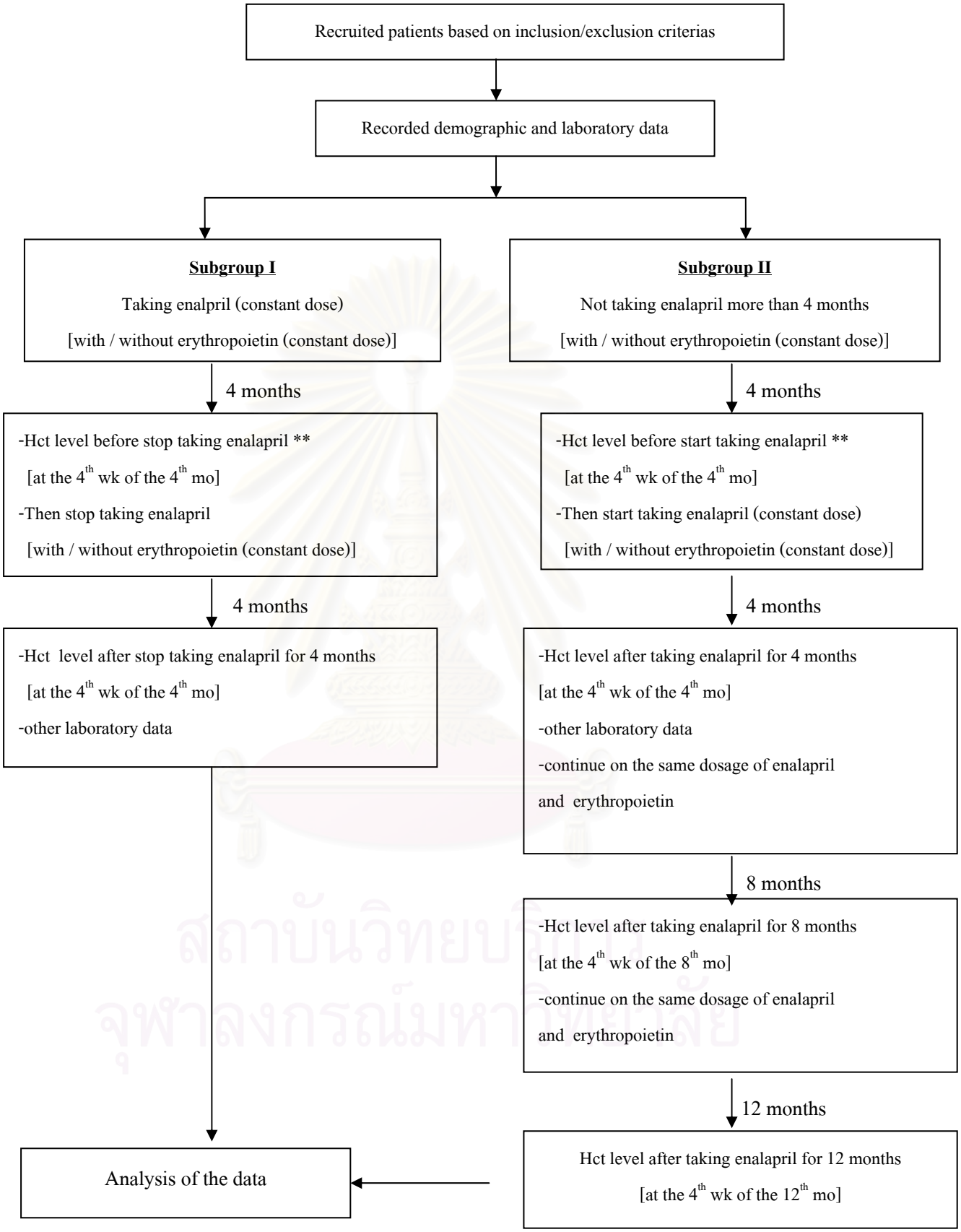


Figure 5.2: Flow chart of the study

CHAPTER IV

RESULTS

1. Demographic data

Fifty-seven patients completed this study, their demographic data were summarized in table 5, the detail characteristics of each individual patient were presented in appendix A.

There were thirty-five men and twenty-two women with a range of age 22-63 years with the mean age equaled to 41.36 ± 11.28 years. The mean duration under hemodialysis treatment at the beginning of the study was 4.91 ± 3.35 years (range 1-15). Mean frequency of hemodialysis was 2.53 times per week (range 2-3). The three most common caused of chronic kidney disease were glomerulonephritis found in 20 patients (35.1%), hypertension found in 9 patients (15.8%) and diabetic nephropathy found in 5 patients (8.8%). Most common comorbid condition was hypertension (57.9%).

Table 5 : Demographic data of the patients

Demographic data	Number of patients N = 57(%)
Sex	
Male	35 (61.4%)
Female	22 (38.6%)
Mean age (yrs) = 41.36 ± 11.28 (range; 22 - 63)	
Mean dry weight (kg) = 52.48 ± 11.04 (range; 33.50 - 95.00)	
Mean height (cm) = 159.71 ± 8.38 (range; 145 - 195)	
Duration of hemodialysis (yrs) = 4.91 ± 3.35 (range; 1-15)	
Frequency of dialysis (times/wk) = 2.53 ± 0.50 (range; 2 - 3)	
Causes of chronic kidney disease	
Glomerulonephritis : Biopsy-proven	
-Focal Segmental Glomerulonephritis	1 (1.8%)
-IgA Nephropathy	1 (1.8%)
-Mesangial proliferative IgM Nephropathy	2 (3.5%)
-Crecentric Glomerulonephritis (RPGN)	1 (1.8%)
Glomerulonephritis : Presumed (no biopsy)	20 (35.1%)
Diabetic Nephropathy	5 (8.8%)
Hypertension	9 (15.8%)
Cystic Kidney Disease	1 (1.8%)
Obstructive Nephropathy, CTIN	4 (7.0%)
Lupus Nephritis	1 (1.8%)
Allograft Dysfunction	1 (1.8%)
Unknown	10 (17.5%)
No data	1 (1.8%)
Comorbid condition	
Chronic Heart Failure (CHF)	1 (1.8%)
Hypertension (HTN)	33 (57.9%)
Acute Myocardial Infarction (AMI) or Unstable Angina (UA) and HTN	3 (5.3%)
CHF and HTN	7 (12.3%)
HTN and DM	1 (1.8%)
AMI or UA and CHF and HTN	1 (1.8%)
AMI or UA and HTN and DM	2 (3.5%)
AMI or UA and CHF and HTN and DM	2 (3.5%)
None	2 (3.5%)
No data	5 (8.8%)

2. Laboratory data

Laboratory data of the patients, before and after study for 4 months, were summarized in table 6. There were no significant changes with regard to laboratory data except for serum potassium (p=0.037). The serum potassium was increased after the patients took enalapril for 4 months. This statistically significant change in serum potassium was hyperkalemia that caused by the adverse effect of enalapril.

Table 6 : Laboratory data of the patients

Serum chemistry	N	Before study (range)		N	After study for 4 months (range)		P-value*
		mean±SD	range		mean±SD	range	
BUN	55	63.19±20.22	30-127	48	63.86±16.79	38-107	0.398
Creatinine	57	11.61±3.60	5.50-24.20	55	10.84±2.58	5.4-18.58	0.107
Uric acid	50	7.32±2.04	2.90-11.70	49	7.44±1.79	4.3-11.4	0.890
Sodium	57	140.71±2.99	132-148	57	139.96±3.24	132-148	0.113
Potassium	57	4.56±0.65	3.2-6.2	57	4.80±0.74	3.28-6.7	0.037
Chloride	57	100.89±3.57	91.80-109	56	101.22±4.62	93-119	0.507
Bicarbonate	57	23.87±4.98	2.5-31	57	24.09±3.43	15-33	0.781
Calcium	56	9.55±1.43	2.64-13.30	55	9.45±1.86	2.4-14.4	0.750
Phosphate	57	4.91±1.78	1.43-11	55	4.92±1.93	0.66-9.2	0.961
Albumin	50	4.19±0.39	3.3-5.4	52	4.10±0.37	2.8-4.7	0.078
SGOT (AST)	16	19.63±12.01	9-45	21	18.95±12.59	4-58	**
SGPT (ALT)	16	18.44±14.44	6-50	21	20.09±24.90	5-125	**
Parathyroid hormone	26	304.39±317.41	5.58-1361	26	475.61±397.08	30.7-1305	0.660
Serum Ferritin	9	634.80±329.44	140.5-1058	12	640.92±549.10	82.1-1610	**
TIBC	7	169.75±106.46	25.08-271	9	255.01±97.54	39-346	**
Serum Iron	6	68.67±16.97	49-92	8	86.63±37.96	13-130	**

* laboratory data comparison before and after taking enalapril

** number of subjects was too small for valid statistical calculation.

3. Effects of enalapril on Hct levels of patients

Hct levels of 57 patients while they did and did not take enalapril were shown in table 7. These 57 patients could be divided into two subgroups. Subgroup I composed of patients who were recruited while they were not taking enalapril, then, enalapril was started and their Hct levels were monitored for the next 4 months and compared with their Hct levels before taking enalapril, the result was shown in table 8. Subgroup II composed of patients who were taking enalapril while they were recruited into the study, then, enalapril was stopped and Hct levels were monitored for the next 4 months since they stopped taking enalapril, these Hct levels were compared and shown in table 9.

The results from table 7, table 8 and table 9, all indicated that Hct levels were statistically significantly lower while taking enalapril.

The percentage of changes were not significantly different between the two subgroups.

Table 7 : Comparison of Hct levels while taking and not taking enalapril.

Hct levels % (mean±SD) N=57		
Take enalapril	Not taking enalapril	P-value
30.11±5.96	27.16±5.88	<0.001

Table 8 : Comparison of Hct levels before taking enalapril and after taking enalapril for 4 months.

Subgroup I patients (N=48)	Hct before start enalapril %(mean±SD)	Hct after taking enalapril for 4 months %(mean±SD)	Percentage of change (mean±SD)	P-value
Patient who were recruited while not taking enalapril then start taking enalapril	29.85±6.05	26.79±5.99	-10.16±9.95*	<0.001

Table 9 : Comparison of Hct levels while taking enalapril and after stop taking enalapril for 4 months.

Subgroup II Patients (N=9)	Hct after stop taking enalapril for 4 months %(mean±SD)	Hct when taking enalapril %(mean±SD)	Percentage of change (mean±SD)	P-value
Patient who were recruited while taking enalapril then stop taking enalapril	31.44±5.59	29.11±5.11	-7.05 ±7.52*	0.025

* comparison percentages of change between subgroup I and subgroup II (p-value =0.377)

4. Effects of dosages of enalapril on Hct levels of patients

In order to see the effects of different dosages of enalapril on Hct levels, the 57 patients included into the study were categorized into 4 subgroups based on the doses of enalapril that they were taking. The result in table 10 indicated that Hct levels of the patients were statistically significantly decreased while taking either dosage of enalapril, even when the dosage was as low as 2.5-5 mg/day. The dosage of 10 mg/day of enalapril caused significantly higher percentage of decrement in Hct levels when compared with the dosage of 2.5-5 mg/day, as shown in table 11. Higher than 10 mg/day dosage of enalapril did not show further decrement in Hct levels. The dosages of enalapril would therefore be categorized into two groups only i.e., lower than than 10 mg/day (low dose) and 10 mg/day or higher (high dose). Comparisons of the effect of high and low doses of enalapril on the percentages of change in Hct level were shown in table 12. The defference was significant at p=0.090.

Table 10 : Effects of different dosages of enalapril on Hct levels.

Dose of enalapril	N (57)	Hct levels % (mean±SD)		Percentage of change (mean±SD)	p-value
		Not taking enalapril	take enalapril		
2.5-5 mg/day	25	29.96±5.54	27.72±5.09	-7.22±7.30	<0.001
10 mg/day	13	28.54±5.24	24.31±5.92	-14.87±12.63	0.002
20 mg/day	12	31.33±6.77	28.08±6.26	-9.85±10.73	0.008
40 mg/day	7	31.43±7.70	28.86±7.31	-8.41±5.86	0.007
ANOVA analysis				0.229	

Table 11 : Comparisons of percentage of changes in Hct levels between different dosages of enalapril

	2.5-5 mg/day	**10 mg/day**	**20 mg/day**	**40 mg/day**
2.5-5 mg/day	-	P=0.023	P=0.387	P=0.697
10 mg/day	-	-	P=0.298	P=0.221
20 mg/day	-	-	-	P=0.749
40 mg/day	-	-	-	-

Table 12 : Effects of high and low doses of enalapril on Hct levels.

Dose of enalapril	N (57)	Hct levels % (mean±SD)		Percentage of change (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
Low dose Enalapril (< 10 mg/day)	25	29.96±5.54	27.72±5.09	-7.23±7.30	<0.001
High dose Enalapril (≥ 10 mg/day)	32	30.22±6.35	26.72±6.48	-11.58±10.83	<0.001
p-value		0.873	0.529	0.090	

5. Effects of erythropoietin on percentage of Hct change caused by enalapril

The inclusion criteria required only that the dosage of erythropoietin should be constant throughout the study, therefore the patients could be included into the study whether or not they were treated with erythropoietin. Table 13 showed that there were statistically significant decrement in Hct levels while taking enalapril in both groups of patients either untreated or treated with erythropoietin. The percentage of decreasing of the group without erythropoietin was higher than the percentage of decreasing of the group with erythropoietin.

To rule out the effect of erythropoietin, only the 30 patients who did not treat with erythropoietin were further studied on the effect of enalapril doses by divided into 2 and 4 subgroups according to the dosages of enalapril that they were taken. The higher dosage of enalapril did not show higher percentage of decrement, except for 10 mg/day subgroups. The results were shown in table 14, 15 and 16.

For 10 mg/day subgroup, there was higher percentage of decrement when compared with other subgroups, especially when compared with 2.5-5 mg/day subgroup, the difference was statistically significant (p=0.051, as shown in table 15). Comparison the percentages of change in Hct levels between higher or equal to 10 mg/day and lower than 10 mg/day dosages of enalapril indicated significant higher decrement for higher dosage at p=0.171 as shown in table 16.

Table 13 : Effects of enalapril on Hct levels with and without erythropoietin.

Total patients	N	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
	(57)	Not taking enalapril	Take enalapril		
Patients without erythropoietin	30	29.33±7.23	25.77±6.86	-11.90±10.87	<0.001
Patients with Erythropoietin	27	30.96±4.10	28.70±4.17	-7.19±7.43	<0.001
P-value		0.307	0.059	P=0.064	

Table 14: Effects of different doses of enalapril on Hct level of patients without erythropoietin.

Dose of enalapril (patients without erythropoietin)	N (30)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
2.5-5 mg/day	13	29.23±6.67	26.38±4.81	-8.77±7.23	0.004
10 mg/day	8	28.13±5.94	22.88±6.81	-18.76±14.82	0.014
20 mg/day	4	29.75±10.81	26.50±10.72	-10.86±13.80	0.005
40 mg/day	5	31.20±9.39	28.20±8.84	-9.92±6.37	0.023
ANOVA analysis				0.337	

Table 15: Comparison between groups of different dosage of enalapril (patients without erythropoietin only)

	2.5-5 mg/day	**10 mg/day**	**20 mg/day**	**40 mg/day**
2.5-5 mg/day	-	P=0.051	P=0.687	P=0.759
10 mg/day	-	-	P=0.396	P=0.238
20 mg/day	-	-	-	P=0.895
40 mg/day	-	-	-	-

Table 16: Effects of high and low doses of enalapril on Hct levels of patients without erythropoietin.

Dose of enalapril (patients without erythropoietin)	N	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
Low dose Enalapril (< 10 mg/day)	13	29.23±6.67	26.38±4.81	-8.77±7.23	0.004
High dose Enalapril (≥ 10 mg/day)	17	29.41±7.83	25.29±8.21	-14.30±12.68	<0.001
p-value		0.947	0.674	0.171	

For the patients who received erythropoietin, the result showed that there were no statistical significant differences in Hct level when patients took or stopped taking enalapril for 4 months, as shown in table 17, 18 and 19. The cross-tab results were grouped in table 20.

Table 17: Effects of enalapril and erythropoietin dose to percentage of change in Hct level.

Dose of enalapril	N (27)	Dose of erythropoietin (unit/kg/wk)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
			Not taking enalapril	Take enalapril		
2.5-5 mg/day	12	66.80±22.85	30.75±4.14	29.17±5.18	-5.57±7.30	0.025
10 mg/day	5	68.24±34.27	29.20±4.44	26.60±3.65	-8.64±3.93	0.012
20 mg/day	8	72.24±39.41	32.13±4.45	28.88±3.18	-9.35±9.92	0.045
40 mg/day	2	69.46±30.34	32.00±1.41	30.50±0.71	-4.64±2.00	0.205
ANOVA analysis					0.337	

Table 18: Comparison between groups of different dosage of enalapril (patients with erythropoietin only)

	2.5-5 mg/day	**10 mg/day**	**20 mg/day**	**40 mg/day**
2.5-5 mg/day	-	P=0.393	P=0.338	P=0.866
10 mg/day	-	-	P=0.884	P=0.245
20 mg/day	-	-	-	P=0.540
40 mg/day	-	-	-	-

Table 19: Effects of high and low doses of enalapril on Hct levels of patients with erythropoietin.

Dose of enalapril	N (27)	Dose of erythropoietin (unit/kg/wk)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
			Not taking enalapril	Take enalapril		
Low dose Enalapril (< 10 mg/day)	12	66.80±22.85	30.75±4.14	29.17±5.18	-5.57±7.30	0.025
High dose Enalapril (≥ 10 mg/day)	15	70.53±34.37	31.13±4.21	28.33±3.29	-8.49±7.51	0.002
p-value		0.749	0.815	0.615	0.319	

Table 20: Effect of enalapril and erythropoietin on percentage of change in Hct level.

Dose of enalapril	Erythropoietin	N (57)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
			No taking enalapril	Take enalapril		
Low dose (<10 mg/d) n=25	Without erythropoietin	13	29.23±6.67	26.38±4.81	-8.77±7.23	0.004
	With erythropoietin	12	30.75±4.14	29.17±5.18	-5.57±7.30	0.025
	p-value		0.505	0.177	0.283	
High dose (≥10 mg/d) n=32	Without erythropoietin	17	29.41±7.83	25.29±8.21	-14.30±12.68	<0.001
	With erythropoietin	15	31.13±4.21	28.33±3.29	-8.49±7.51	0.002
	p-value		0.453	0.190	0.132	

6. Effects of dosages of erythropoietin on percentage of change in Hct level by enalapril

The patients who categorized to low dose enalapril group and high dose enalapril group, each group also was categorized to patients with and without erythropoietin. In patients with erythropoietin, the patients were divided into two subgroups, low dose erythropoietin (patients who received erythropoietin 20-80 unit/kg/wk) and high dose erythropoietin (patients who received erythropoietin more than or equal to 80 unit/kg/wk). The Hct level of these subgroups has shown in table 17-20 respectively. Similarly, the patients categorized by the dosages of enalapril, there were also no statistically significant different in the percentage of decreasing in all dosages of enalapril, either high dose enalapril or low dose enalapril (the results were shown in table 21,22 and 23).

Table 21: Effect of erythropoietin dose on percentage of change in Hct level.

Dose of erythropoietin	N (27)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
< 80 unit/kg/wk	15	29.73±3.84	27.73±3.73	-6.62±6.18	0.001
≥ 80 unit/kg/wk	12	32.50±4.03	29.92±4.52	-7.90±8.98	0.018
p-value		0.081	0.181	0.665	

Table 22: Low dose enalapril (< 10 mg/day)

Dose of erythropoietin	N	Hct levels % (mean±SD)		Percentage of changes	p-value
	12	Not taking enalapril	Take enalapril	(mean±SD)	
20-80 unit/kg/wk	7	30.57±4.04	28.71±4.46	-6.27±6.13	0.026
≥ 80 unit/kg/wk	5	31.00±4.74	29.80±6.57	-4.58±9.39	0.388
p-value		0.869	0.739	0.713	

Table 23: High dose enalapril (≥ 10 mg/day)

Dose of erythropoietin	N	Hct levels % (mean±SD)		Percentage of changes	p-value
	15	Not taking enalapril	Take enalapril	(mean±SD)	
20-80 unit/kg/wk	8	29.00±3.78	26.88±2.99	-6.93±6.63	0.034
≥ 80 unit/kg/wk	7	33.57±3.41	30.00±2.94	-10.27±8.57	0.031
p-value		0.030	0.063	0.410	

7. Effects of baseline Hct level on the percentage of Hct change caused by enalapril

Baseline Hct of patients were categorized to 4 subgroups (A, B, C and D) to find the correlation between the percentages of Hct change and baseline Hct levels. In total patients, The results indicated that the patients who had baseline Hct below 25% (subgroup A) had the highest percentage of decrement, but there were no statistically significant different compared with other baseline Hct of patients both in the total patients and the patients who received erythropoietin. In contrast, the patients who did not receive erythropoietin, the percentages of decrement were high in almost baseline Hct (subgroup A, C and D). The correlation between the percentages of Hct change and baseline Hct levels, were shown in table 24, 25 and 26.

Table 24 : Comparisons of the percentage of change in Hct levels caused by enalapril based on baseline Hct levels (total patients, n=57).

Baseline Hct levels (%)	N (57)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
A; Hct <25	14	23.00±1.88	20.07±2.46	-12.45±10.73	0.001
P value of A vs B		<0.001	<0.001	0.138	
B; 26<Hct≤29	14	27.64±1.15	25.57±1.55	-7.38±6.17	0.001
P value of B vs C		<0.001	0.009	0.678	
C; 30<Hct≤33	14	31.43±1.22	28.57±3.65	-8.91±12.12	0.020
P value of C vs D		<0.001	0.001	0.796	
D; Hct >33	15	37.80±3.80	33.93±3.77	-9.93±8.81	0.001
P value of D vs A		<0.001	<0.001	0.494	

Table 25 : Comparisons of the percentage of change in Hct levels caused by enalapril based on baseline Hct levels (patients received erythropoietin , n=27).

Baseline Hct levels (%)	N (27)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
A; Hct <25	3	23.67±1.53	20.67±0.58	-12.40±6.81	0.095
P value of A vs B		0.004	0.001	0.235	
B; 26<Hct≤29	6	27.83±1.33	26.00±1.67	-6.47±6.30	0.058
P value of B vs C		<0.001	0.001	0.373	
C; 30<Hct≤33	9	31.22±1.20	29.89±1.62	-4.30±2.78	0.002
P value of C vs D		<0.001	0.076	0.233	
D; Hct >33	9	35.22±1.92	32.00±2.92	-8.82±10.59	0.039
P value of D vs A		<0.001	<0.001	0.601	

Table 26 : Comparisons of the percentage of change in Hct levels caused by enalapril based on baseline Hct levels (patients did not receive erythropoietin , n=30).

Baseline Hct levels (%)	N (30)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	p-value
		Not taking enalapril	Take enalapril		
A; Hct <25	11	22.82±1.99	19.91±2.77	-12.46±11.85	0.006
P value of A vs B		<0.001	<0.001	0.356	
B; 26<Hct≤29	8	27.50±1.07	25.25±1.49	-8.06±6.41	0.010
P value of B vs C		<0.001	0.630	0.210	
C; 30<Hct≤33	5	31.80±1.30	26.20±5.22	-17.21±18.11	0.106
P value of C vs D		<0.001	0.002	0.487	
D; Hct >33	6	41.67±2.16	36.83±3.06	-11.59±5.69	0.004
P value of D vs A		<0.001	<0.001	0.869	

8. Effects of duration of enalapril taken on Hct levels

Only thirteen patients who received constant dose of enalapril and constant dose of erythropoietin (including those who did not take any erythropoietin and packed red cell) throughout 12 months, therefore, their Hct levels could be monitored every 4 months for 12 months and the results were recorded in table 27. The results showed that Hct levels were decreasing at 4 and 8 months after taking enalapril, then, the Hct levels seem to start adjusting up at 12 months.

From 13 patients who took enalapril up to 12 months, the patients were categorized to patients who taken enalapril without erythropoietin (n=9) and with erythropoietin (n=4). Hct levels and the percentage of decreasing in Hct levels of all patients, and patients in both subgroups were shown in figure 5-10.

Table 27 : Hct levels before taking enalapril and after taking enalapril for 4, 8 and 12 months. (N=13)

Case No.	Dose of erythropoietin (Unit/kg/wk)	Dose of erythropoietin (Unit/wk)	Dose of enalapril (mg/day)	Hct before taking enalapril (%)	Hct after taking enalapril for 4 months (%)	% change at 4 months	Hct after taking enalapril for 8 months (%)	% change at 8 months	Hct after taking enalapril for 12 months (%)	% change at 12 months
A04	.00	0	5.00	26.00	25.00	-3.85	30.00	15.38	32.00	23.08
A08	.00	0	5.00	24.00	23.00	-4.17	25.00	4.17	26.00	8.33
A09	.00	0	5.00	21.00	19.00	-9.52	18.00	-14.29	17.00	-19.05
A11	.00	0	5.00	28.00	26.00	-7.14	27.00	-3.57	27.00	-3.57
A14	.00	0	10.00	40.00	37.00	-7.50	20.00	-50.00	28.00	-30.00
A21	.00	0	20.00	29.00	25.00	-13.79	30.00	3.45	31.00	6.90
A24	.00	0	40.00	28.00	28.00	0.00	33.00	17.86	35.00	25.00
A25	.00	0	40.00	25.00	21.00	-16.00	22.00	-12.00	20.00	-20.00
A26	.00	0	40.00	43.00	38.00	-11.63	31.00	-27.91	27.00	-37.21
A34	48.00	3000	40.00	31.00	30.00	-3.23	27.00	-12.90	29.00	-6.45
A38	77.67	4000	5.00	31.00	31.00	0.00	29.00	-6.45	33.00	6.45
A40	86.02	4000	5.00	33.00	33.00	0.00	30.00	-9.09	33.00	0.00
A47	101.27	4000	10.00	34.00	30.00	-11.76	35.00	2.94	35.00	2.94
Mean ± SD	-	-	-	30.23±6.21	28.15±5.77	-6.81±5.45	27.46±5.03	-7.11±17.95	28.69±5.47	-3.35±18.89

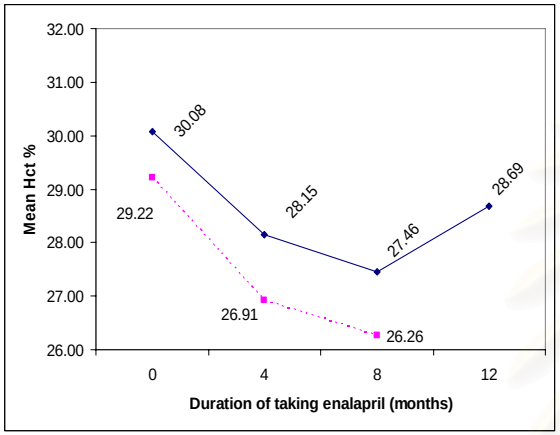


Figure 6.1: Hct levels after taking enalapril for 12 months (total patients, n=13)compared with 8 months (total patients, n=23)

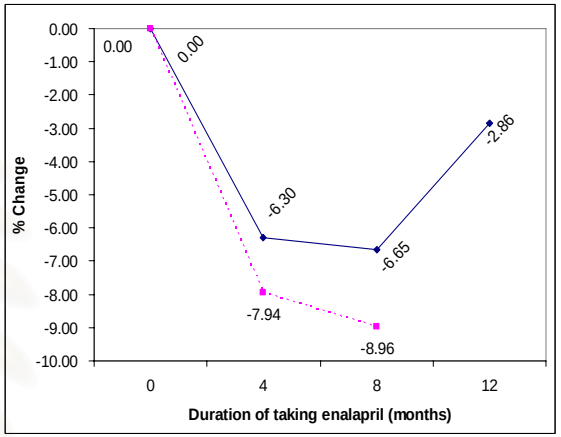


Figure 6.2: The percentage of decreasing after taking enalapril for 12 months (total patients, n=13)compared with 8 months (total patients, n=23)

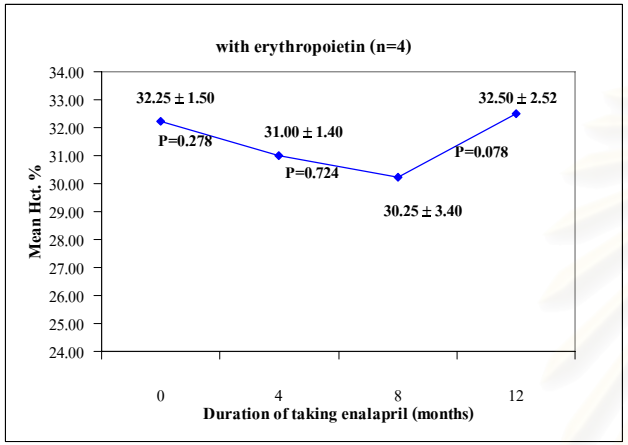


Figure 7: Hct levels after taking enalapril for 12 months (patients received erythropoietin)

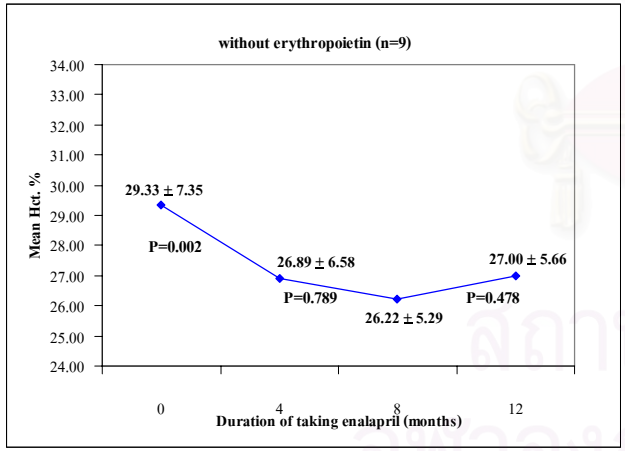


Figure 8: Hct levels after taking enalapril for 12 months (patients did not receive erythropoietin)

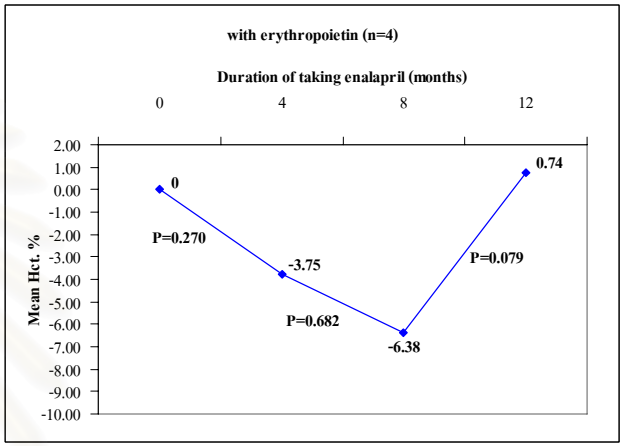


Figure 9: The percentage of decreasing after taking enalapril for 12 months (patients received erythropoietin)

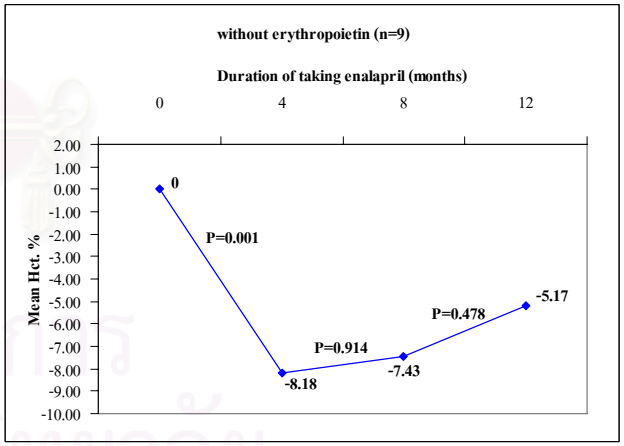


Figure 10: The percentage of decreasing after taking enalapril for 12 months (patients did not received erythropoietin)

There were 23 patients whose data could be monitored up to 8 months but could not be monitored to 12 months due to various reasons. The data of each patient was shown in table 28. The decreasing of Hct levels and the percentage of change were shown in figure 11-14.

Table 28 : Hct levels before taking enalapril and after taking enalapril for 4 and 8 months. (N=23)

Case No.	Dose of erythropoietin (Unit/kg/wk)	Dose of erythropoietin (Unit/wk)	Dose of enalapril (mg/day)	Hct before taking enalapril (%)	Hct after taking enalapril for 4 months (%)	% change at 4 months	Hct after taking enalapril for 8 months (%)	% change at 8 months
A04	0	0	5	26	25	-3.85	30	15.38
A05	0	0	5	30	28	-6.67	28	-6.67
A07	0	0	5	21	20	-4.76	21	0.00
A08	0	0	5	24	23	-4.17	25	4.17
A09	0	0	5	21	19	-9.52	18	-14.29
A11	0	0	5	28	26	-7.14	27	-3.57
A14	0	0	10	40	37	-7.50	20	-50.00
A16	0	0	10	28	23	-17.86	23	-17.86
A18	0	0	10	28	24	-14.29	20	-28.57
A21	0	0	20	29	25	-13.79	30	3.45
A24	0	0	40	28	28	0.00	33	17.86
A25	0	0	40	25	21	-16.00	22	-12.00
A26	0	0	40	43	38	-11.63	31	-27.91
A27	20.83	1000	10	30	28	-6.67	26	-13.33
A33	46.51	2000	20	30	29	-3.33	28	-6.67
A34	48	3000	20	31	30	3.45	27	-6.90
A35	52.63	2000	5	24	20	-16.67	21	-12.50
A38	77.67	4000	5	31	31	0.00	29	-6.45
A40	86.02	4000	5	33	33	0.00	30	-9.09
A43	90.91	4000	10	29	25	-13.79	23	-20.69
A44	90.91	4000	40	33	31	-6.06	32	-3.03
A45	93.02	4000	5	27	25	-7.41	25	-7.41
A47	101.27	4000	10	34	30	-11.76	35	2.94
Mean ± SD	-	-	-	29.26±5.19	26.91±5.09	-8.31±5.50	26.26±4.66	-11.26±12.73

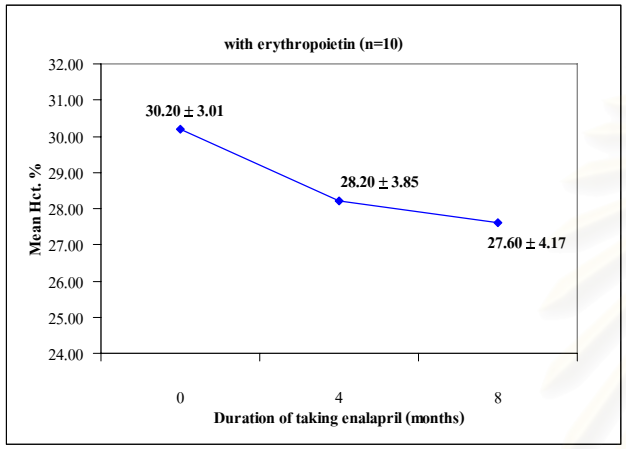


Figure 11: Hct levels after taking enalapril for 8 months (patients received erythropoietin)

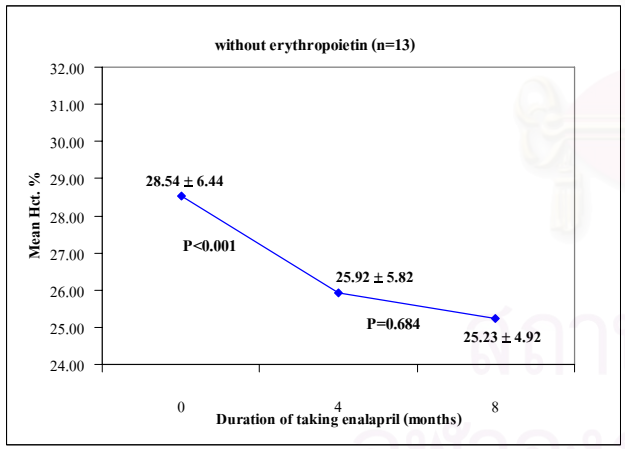


Figure 12: Hct levels after taking enalapril for 8 months (patients did not receive erythropoietin)

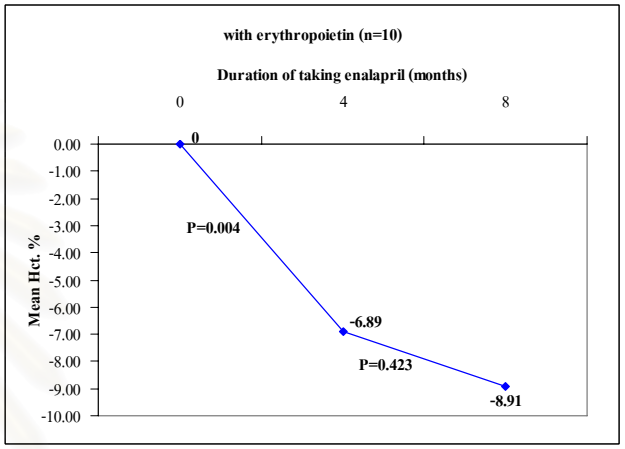


Figure 13: The percentage of decreasing after taking enalapril for 8 months (patients received erythropoietin)

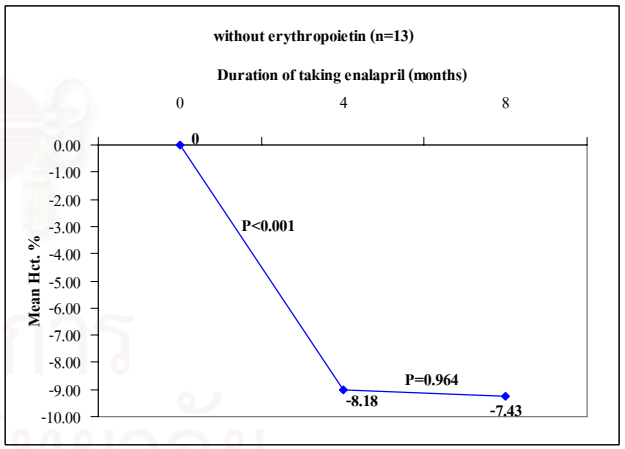


Figure 14: The percentage of decreasing after taking enalapril for 8 months (patients did not receive erythropoietin)

There were 34 patients whose data could not be monitored throughout the 12 months since their conditions were changed, either the dose of enalapril and/or the dose of erythropoietin were changed or packed red cell had to be given to the patients. Majority of the patients who did not receive erythropoietin at baseline were excluded because their Hct levels were too low and erythropoietin or packed red cell had to be given while some were excluded due to requirement to change in the dosage of enalapril. Majority of the patients who had been given erythropoietin at baseline were excluded due to change in the dosage of enalapril while minority were excluded due to requirement to change in the dosage of erythropoietin given or packed red cell had to be added. The details were shown in table 29, 30 and 31.

Table 29: Reasons for termination of the monitoring before the end of 12 months (patients did not receive erythropoietin at baseline; n=17).

Case number	Enalapril Dose (mg/day)	Erythropoietin Dose (unit/wk)	Months	Enalapril Dose Changes	Erythropoietin Dose Changes or add PRC	Hct (%)
A01	2.5	0	7	Off enalapril		22
A02	5	0				
A03	5	0	6		8000 unit/wk	
A05	5	0	9	Off enalapril		28
A06	5	0	5	5 to 40 mg/d		
			6		4000 unit/wk	
A07	5	0	12		PRC 2 unit	16 to 25
A10	5	0	5	Off enalapril		28
A12	10	0	5		8000 unit/wk	16
A13	10	0	5		PRC 2 unit	18 to 24
A15	10	0	6	10 to 20 mg/d		
A16	10	0	11		Parenteral iron injection	
A17	10	0	5		PRC 2 unit	16 to 22
A18	10	0	10		8000 unit/wk	20
A19	20	0	5	Off enalapril		42
A20	20	0	6		PRC 2 unit	20 to 22
A22	20	0	7		PRC 2 unit	17 to 23
A23	20	0	7		PRC 2 unit	18 to 23.5

Table 30: Reasons for termination of the monitoring before the end of 12 months (patients received erythropoietin at baseline; n=17).

Case number	Enalapril Dose (mg/day)	Erythropoietin Dose (unit/wk)	Months	Enalapril Dose Changes	Erythropoietin Dose Changes or add PRC	Hct (%)
A27	10	1000	9	10 to 20 mg/d		
A28	5	1500	5		Parenteral iron injection	
A29	20	2000	5		Unstable EPO	
A30	20	2000	8	*		
A31	5	2000	6	Off enalapril		27
A32	20	2000	5	20 to 40 mg/d		
A35	5	2000	10		Off EPO (BP ↑)	
A36	5	4000	6	5 to 10 mg/d		
A37	20	4000	6	*		
A39	10	4000	9	10 to 20 mg/d		
A41	5	4000	5		PRC 2 unit	21 to 30
A42	5	4000	8	Off enalapril		24
A43	10	4000	9	Off enalapril		23
A44	40	4000	9		Off EPO	
A45	5	4000	9		Unstable EPO	
A46	20	4000	5	*		
A48	20	4000	7	*		

* This study ended before the process of 12 months monitoring.

Table 31: Comparisons of the reasons for termination of the monitoring before the end of 12 months between patients who received and did not received erythropoietin.

Reason of termination	With erythropoietin (n=17)	Without erythropoietin (n=17)
Enalapril dose change	4	3
Off enalapril	3	4
Erythropoietin dose change	2 (unstable)	4 (start)
Off erythropoietin	2	-
Blood transfusion	1	6
Parenteral iron injection	1	1
Study ended	4	-

9. Other factors that may influence the change in Hct levels.

1. Sex

The percentage of decreasing in Hct levels caused by enalapril in male and female were not significantly different either when considered the total patients or when categorized into two subgroups with and without erythropoietin. The results were shown in table 32.

Table 32: Comparisons of the percentage of change in Hct levels caused by enalapril between male and female.

Sex		N	Hct levels % (mean±SD)		Percentage of change (mean±SD)	P-value
			Not taking enalapril	Take enalapril		
Total	Male	35	30.09±6.67	26.77±6.44	-10.80±10.81	<0.001
	Female	22	30.05±4.78	27.77±4.95	-7.57±7.52	<0.001
P-value			0.980	0.536	0.496	
EPO	Male	10	30.00±3.80	28.10±3.07	-5.95±6.59	0.027
	Female	17	31.41±4.32	29.06±4.75	-7.52±8.37	0.004
P-value			0.400	0.574	0.617	
Without EPO	Male	25	30.12±7.59	26.24±7.36	-12.74±11.65	<0.001
	Female	5	25.40±3.21	23.40±2.70	-7.72±4.09	0.022
P-value			0.187	0.407	0.355	

2. Age

The patients were categorized into four groups based on their ages that were shown in table 32. The percentage of decreasing in Hct levels caused by enalapril were not statistically different between four groups with different ages. The results were shown in table 33, 34 and 35.

Table 33: Comparisons of the percentage of change in Hct levels caused by enalapril among patients with different aged groups (total patients, n=57).

Age (years)	N	Hct levels % (mean±SD)		Percentage of change (mean±SD)	P-value
	57	Not taking enalapril	Take enalapril		
A; < 30	8	29.75±6.88	26.00±7.46	-13.28±11.67	0.022
P value of A vs B		0.339	0.545	0.495	
B; 30≤ x <40	16	27.56±4.13	24.63±3.63	-9.71±11.98	0.009
P value of B vs C		0.015	0.011	0.982	
C; 40≤ x <50	21	31.62±5.18	28.57±4.99	-9.64±6.29	<0.001
P value of C vs D		0.816	0.907	0.408	
D; x ≥ 50	12	31.08±7.98	28.83±7.77	-7.26±10.03	0.022
P value of D vs A		0.704	0.428	0.234	

Table 34: Comparisons of the percentage of change in Hct levels caused by enalapril between patients with different aged groups (patients received erythropoietin at baseline, n=27).

Age (years)	N	Hct levels % (mean±SD)		Percentage of change (mean±SD)	P-value
	27	Not taking enalapril	Take enalapril		
A; < 30	4	34.75±4.11	31.00±2.94	-10.04±11.63	0.205
P value of A vs B		0.020	0.045	0.770	
B; 30≤ x <40	8	28.13±3.79	25.75±4.03	-8.49±6.49	0.008
P value of B vs C		0.023	0.039	0.786	
C; 40≤ x <50	11	32.09±3.14	29.64±3.53	-7.69±6.03	0.002
P value of C vs D		0.249	0.962	0.061	
D; x ≥ 50	4	29.75±3.86	29.75±5.25	-0.32±6.62	1.000
P value of D vs A		0.127	0.692	0.196	

Table 35: Comparisons of the percentage of change in Hct levels caused by enalapril between patients with different aged groups (patients did not received erythropoietin at baseline, n=30).

Age (years)	N	Hct levels % (mean±SD)		Percentage of change (mean±SD)	P-value
	30	Not taking enalapril	Take enalapril		
A; < 30	4	24.75±5.19	21.00±7.39	-16.52±12.42	0.087
P value of A vs B		0.462	0.413	0.560	
B; 30≤ x <40	8	27.00±4.63	23.50±3.02	-10.92±16.18	0.106
P value of B vs C		0.171	0.124	0.879	
C; 40≤ x <50	10	31.10±6.94	27.40±6.20	-11.78±6.16	0.001
P value of C vs D		0.870	0.790	0.788	
D; x ≥ 50	8	31.75±9.60	28.38±9.07	-10.73±9.90	0.009
P value of D vs A		0.209	0.192	0.399	

3. Duration of hemodialysis

Patients were categorized into three groups based on the duration of hemodialysis. The percentage of decreasing in Hct levels caused by enalapril were nearly the same. The results were shown in table 36, 37 and 38.

Table 36: Comparisons of the percentage of change in Hct levels caused by enalapril among patients with different duration of hemodialysis (total patients, n=57)

Duration of hemodialysis (years)	N (57)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
		Not taking enalapril	Take enalapril		
A ; 1-3	22	29.45±6.35	26.95±6.59	-8.63±10.06	0.001
p value of A vs B		0.627	0.414	0.617	
B ; 4-6	19	28.58±4.85	25.47±4.53	-10.28±10.99	0.002
p value of B vs C		0.034	0.031	0.923	
C ; 7-15	16	32.69±6.12	29.44±5.88	-9.96±8.06	<0.001
p value of C vs A		0.124	0.238	0.664	

Table 37: Comparisons of the percentage of change in Hct levels caused by enalapril among patients with different duration of hemodialysis (patients received erythropoietin at baseline, n=27)

Duration of hemodialysis (years)	N (27)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
		Not taking enalapril	Take enalapril		
A ; 1-3	9	33.00±3.24	31.22±2.49	-4.81±9.44	0.180
p value of A vs B		0.007	0.007	0.450	
B ; 4-6	12	28.33±3.63	26.25±4.37	-7.58±7.06	0.004
p value of B vs C		0.026	0.097	0.709	
C ; 7-15	6	32.83±3.76	29.83±3.31	-8.88±6.32	0.023
p value of C vs A		0.928	0.369	0.373	

Table 38: Comparisons of the percentage of change in Hct levels caused by enalapril among patients with different duration of hemodialysis (patients did not receive erythropoietin at baseline, n=30)

Duration of hemodialysis (years)	N (30)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
		Not taking enalapril	Take enalapril		
A ; 1-3	13	27.00±6.89	24.00±6.99	-11.27±9.95	0.001
p value of A vs B		0.542	0.962	0.523	
B ; 4-6	7	29.00±6.78	24.14±4.81	-14.93±15.21	0.054
p value of B vs C		0.323	0.126	0.476	
C ; 7-15	10	32.60±7.38	29.20±7.16	-10.61±9.22	0.002
p value of C vs A		0.075	0.095	0.873	

4. Frequency of hemodialysis

The frequency of hemodialysis either 2 times per week or 3 times per week did not influence the effect of enalapril on Hct levels significantly. The results were shown in table 39, 40, and 41.

Table 39: Comparisons of the percentage of change in Hct levels caused by enalapril between patients categorized by frequency of hemodialysis (total patients, n=57).

Frequency of hemodialysis	N (57)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
		Not taking enalapril	Take enalapril		
2 times/wk	27	28.26±6.16	25.33±5.69	-10.20±8.97	<0.001
3 times/wk	30	31.70±5.37	28.80±5.65	-8.97±10.49	<0.001
P-value		0.028	0.025	0.638	

Table 40 : Comparisons of the percentage of change in Hct levels caused by enalapril between patients categorized by frequency of hemodialysis (patients received erythropoietin at baseline, n=27).

Frequency of hemodialysis	N (27)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
		Not taking enalapril	Take enalapril		
2 times/wk	12	30.00±4.16	27.17±4.22	-9.40±7.88	0.005
3 times/wk	15	31.60±4.09	29.93±3.83	-4.97±7.15	0.020
P-value		0.325	0.086	0.139	

Table 41: Comparisons of the percentage of change in Hct levels caused by enalapril between patients categorized by frequency of hemodialysis (patients did not receive erythropoietin at baseline, n=30).

Frequency of hemodialysis	N (30)	Hct levels % (mean±SD)		Percentage of changes (mean±SD)	P-value
		Not taking enalapril	Take enalapril		
2 times/wk	15	26.87±7.22	23.87±6.40	-10.84±9.99	0.001
3 times/wk	15	31.80±6.56	27.67±6.98	-12.97±11.94	0.001
P-value		0.060	0.131	0.600	

CHAPTER V

DISCUSSION

There were 57 hemodialysis patients enrolled and completed this study. Most patients were male, the age widely from 22 to 63 years old. Most patients dialysed 3 times a week. Duration of hemodialysis ranged from 1-15 years. The most common cause of chronic kidney disease (CKD) of patients in this study was glomerulonephritis. The patients have many complications such as chronic heart failure, acute myocardial infarction or unstable angina and most of them have hypertension.

To reduce the confounding factors, approximately sixteen variables of serum chemistries (blood urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, bicarbonate, calcium, phosphate, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), intact parathyroid hormone, ferritin, total iron-binding capacity (TIBC), and iron) were monitored before the study and after the study for 4 months. There were no significantly difference in most serum chemistries before and after the study, except for serum potassium which might be related to the adverse effect of enalapril; hyperkalemia. For AST, ALT, serum ferritin, TIBC, and serum iron, no statistical calculation could be performed because the number of subjects whose data were observed was too small.

Effects of enalapril on Hct levels of patients

The results obtained, as shown in table 7, 8 and 9, indicated that the use of enalapril, an angiotensin-converting enzyme inhibitor (ACEI) in hemodialysis patients significantly decreased Hct levels as compared to the time that they did not use enalapril. ACEI is a strong inhibitor of angiotensin II production while angiotensin II is

necessitated for erythropoietin formulation, then, in turn decreasing red cell production. The Hct levels decreased significantly after consumed enalapril for 4 months as compared to the past 4 months before starting enalapril. At the same time, for those patients who were started the observation while they were on enalapril for at least 4 months, then, enalapril was stopped, after enalapril was withdrawn for 4 months, the Hct levels increased significantly. There results implied that the inhibition of angiotensin II production caused by enalapril was reversible and only temporary, once the drug was moved out of the body, the effect was discontinued. However, 4 months period of monitoring time was set in order to account for the life cycle of Hct and to ensure the steady state condition.

The results from this study were similar to those reportedly by Erturk *et al.* They studied the Hct levels of 23 patients after stop taking enalapril 10-40 mg/day (n=19), captopril 25-75 mg/day (n=3), and perindopril 2 mg/day (n=1) for 1 and 3 years. They found that withdrawal of ACEI from hypertensive chronic hemodialysis patients who were also receiving erythropoietin resulted in increasing in Hct levels [2]. Several previous studies monitored the influence of enalapril on erythropoietin requirements to control the desired Hct levels [1,4-6]. Albitar *et al*. evaluated prospective, non-randomized, controlled trial follow up 1 years in 60 erythropoietin treated HD patients who were taken enalapril (n=20, 10 and 20 mg/day, nifedipine 40 mg (n=20), and control groups (erythropoietin alone, n=20), they concluded that high-dose enalapril increased erythropoietin requirement [1]. Similarly, Matsumura *et al*. examined retrospective study in 108 HD patients who received erythropoietin alone (n=59) and received erythropoietin with ACE inhibitors (n=49; 31:enalapril, 10:imidapril, 2:captopril, 2:cilazapril, 2:benazepril and 2:temocapril), the data suggested that ACE inhibitors administration increased erythropoietin maintenance doses in HD patients [6]. In addition, Dhondt *et al*. searched for cross-sectional study in 49 HD patients who received erythropoietin alone (n=27) and received erythropoietin with ACE inhibitors (n=22; 16:lisinopril 2.5-20 mg/day, 4:captopril

12.5-37.5 mg/day, 1:perindopril 4 mg/day and 1:ramipril 2.5 mg/day) for 3 months, the study demonstrated that more exogenous erythropoietin was required in ACE inhibitors treated chronic HD patients to achieve the same mean hematocrit of 33% [5].

Hirakata *et al.* studied the effect of captopril in chronic hemodialysis patients who had been taken captopril for 1.5 months, then, stopped taking [4]. The results showed that angiotensin II and angiotensin converting enzyme were reduced significantly. Hemoglobin, hematocrit, red blood cell, reticulocyte count, and plasma erythropoietin concentration were also reduced significantly.

Among the 57 patients included into the study, three of them had their Hct levels higher after taking enalapril for 4 months. Two of these patients received hig dose of erythropoietin during 4-8 months before taking enalapril (study point) and one of them received packed red cell during 4-8 months before taking enalapril (study point). Therefore, the dosage of erythropoietin or packed red cell might need to be kept stable for more than 4 months to be able to completely get away from these confounding factors.

Effects of dosages of enalapril on Hct levels of patients

The results shown in table 10-12 indicated that higher dosage of enalapril showed significantly higher effect on the decrement in Hct levels when compared between the group of patients who consumed 2.5-5 mg/day to the group who consumed 10 mg/day. However, for the group who consumed 20 mg/day or 40 mg/day of enalapril, even though the percentages of decrement were slightly higher than those obtained from patients who consumed 2.5-5 mg/day, but these differences were not statistically significant at α=0.05 and the mean percentages of decrement in Hct levels were not higher than those caused by 10 mg/day of enalapril. This might imply that the effect caused by enalapril had some limitation or could become saturated. On the other hand, several confounding factors might involve since the patients compared were from different groups, their conditions could be different. For example, the baseline

Hct levels of the patients who received 40 mg/day of enalapril were higher than those of the patients who received lower doses of enalapril. Although we observed clinical data of the patient backward for at least 4 months and ensured that the dosage of erythropoietin was kept constant throughout the studied period, however, if the dosage had been adjusted right away beyond the past 4 months, it would not be recorded. Several factors [29] which might influence the results, such as, serum parathyroid hormone and serum ferritin had not been monitored and recorded. Since this is a retrospective study, therefore, these recommended data were missing. Besides, the number of patients recruited in each dosage group, especially the higher dosages, i.e., 20 mg/day and 40 mg/day, were much too small for definite conclusion.

Effects of erythropoietin on percentage of Hct change caused by enalapril

When the total 57 patients included into the study were categorized into two subgroups, with and without treatment with erythropoietin. The results as shown in table 13 indicated that Hct levels before taking enalapril and after taking enalapril were statistically significant difference in both groups ($p<0.001$). However, the effect of enalapril could be seen more prominently in patients who were not treated with erythropoietin, since the percentage of decreasing in Hct levels caused by enalapril in patients who were not treated with erythropoietin was significantly higher than in patients who were treated with erythropoietin ($p=0.064$). From table 17 and 18, the results showed that there were no statistically significant different in the decrement of percentage on Hct levels in patients with many enalapril doses. These results implied that erythropoietin has direct effect on Hct levels. The results shown in table 16 and 19 pointed out that the influence of erythropoietin on the Hct decrement effect of enalapril was more distinct at higher dosage of enalapril. This was similar to the study by Hayashi *et al.*, they suggested that the inhibitory of angiotensin-converting enzyme was most apparent when high dose of ACE inhibitors was administered together with low dose of erythropoietin in hemodialysis patients [12].

Effects of dosages of erythropoietin on percentage of change in Hct level by enalapril

When the patients who received erythropoietin were further categorized into two subgroups according to the dosage of erythropoietin consumed, no noticeable different between the two subgroups could be observed, as shown in table 21, 22 and 23. Higher dosage of erythropoietin should result in lower changed in the percentage of Hct levels caused by enalapril, however, in our cases, there were 3 patients who were treated with erythropoietin more than 100 unit/kg/wk and enalapril $\geq$ 10 mg/day but the percentage of decreasing in Hct levels were higher than others. These 3 patients who consumed high dosage of erythropoietin had their baseline Hct levels which were quite high when compared the other patients, therefore the percentage of decrement in Hct levels might not totally be caused by enalapril, many other factors could also influence decrement in Hct levels, such as, the erythropoietin doses changes, received packed red cell, and parenteral iron injection on 8 months before the point of the study. This study recruited the patients by controlling these factors for 4 months before the point of the study, so our results had some these confounding factors.

Effects of baseline Hct levels on the percentage of Hct change caused by enalapril

From table 24, 25 and 26 indicated that the percentage of Hct decrement did not correlated with baseline Hct levels. The correlation could not be detected whether the patients were received erythropoietin or did not receive erythropoietin. The percentage of Hct changes were not significantly different between patients who had low baseline Hct levels or high baseline Hct levels. The effect of enalapril on Hct levels, therefore, did not depend on baseline Hct levels, but might depend on hemodialysis status, or individual response of the patients.

Effects of duration of enalapril taken on Hct levels

The results from 13 patients who could continue on the same dose of enalapril and erythropoietin throughout 12 months as shown in table 27 and figure 5-10 indicated that enalapril caused significantly decrement in Hct level at 4 months and slightly further decrement was continue on at 8 months, however, at 12 months, the effect of enalapril on erythropoietin formation should reach its maximum or saturated, the patients seem to be tolerant or able to somewhat regulate or adjusting their biological substances, so the erythropoietin formation process started to slightly reverse back. This might be the reason that many previous studies were controversial. Charytan *et al*. studied the effect of enalapril on erythropoietin dosage requirement for 7 months [10], while Cruz *et al*. and Albu Alfa *et al.* studied for 4 months [13, 11], and Hayashi *et al.* studied for 3 months [12], their results showed that enalapril had no effect on erythropoietin dosage requirements. From this study, we suggested that the time to study the effect of enalapril on Hct levels in HD patients should be monitored every 4 months for at least 8-12 months in order to find the clearer and maximum effect of enalapril. The duration of effect of enalapril were more apparent in the 23 patients who could continue on the same dose of enalapril and erythropoietin throughout 8 months especially in the patients who did not receive erythropoietin, as shown in table 28 and figure 11-14.

There were only 13 patients out of the 57 patients enrolled in the study who could continue on the same dosage of enalapril and erythropoietin (including those who did not treat with erythropoietin) throughout 12 months. This was not surprising since enalapril caused decrement in Hct levels, many patients would required increment in the dosage of erythropoietin or required to give packed red cell or the dose of enalapril was required to change or stopped taking enalapril (changed to other hypertensive drug). Majority of patients who received erythropoiesis at baseline were dropped out due to change in the dosage of enalapril while majority of patients who did

not receive erythropoietin at baseline required treatment with erythropoietin or given packed red cell, as shown in table 29, 30 and 31.

Other factors that may influence the change in Hct levels

Sex

The effect of enalapril on the decrement in Hct levels were significantly in both sex but were not statistically significantly different between sex. However, the mean percentage of change was more distinct in the male group especially in patients who were not receiving erythropoietin. The baseline Hct level of female was slightly lower than that of the male in the non-treatment with erythropoietin group. In natural, it is normal that female has lower Hct level than male[35].

Age

Enalapril caused significant decrement in Hct levels in patients in any age. The percentages of decrement was higher in patients who younger than 30 years in both patients who received and did not received erythropoietin. This might due to the major causes of their chronic kidney disease were glomerulonephritis which the causes are no etiology. The patients who were glomerulonephritis, often received immunosuppressant for treatment for while until further hemodialysis. Anemia of these patients and the response to erythropoietin might be affected from this factor.

Duration of hemodialysis

The percentages of decreasing in Hct levels caused by enalapril were nearly the same, no matter how long the patients had been on hemodialysis ranging from 1-15 years. This indicated that the timing which patients on hemodialysis did not affect the effect of enalapril on Hct level.

However, the baseline Hct level of patients who were on hemodialysis for 7-15 years was higher than the patients who were on hemodialysis for only 1-3 years which

could be seen more clearly for patients who were not receiving erythropoietin. The paitents who could survive on hemodialysis for a longer time, could be more stabilize.

Frequency of hemodialysis

The frequency of hemodialysis either 2 times or 3 times affect the effect of enalapril of enalapril in decreasing Hct levels, the percentages of decreasing in Hct levels were similar and the Hct levels were all statistically significantly decreasing from the time when they did not consume enalapril. The baseline Hct levels of the patients who were on hemodialysis 3 times per weeks were significantly higher than the patients who were on hemodialysis 2 times per week. This means that more adequacy of dialysis will cause better response to erythropoietin and in turn higher level of Hct.

CHAPTER VI

CONCLUSION

1. Enalapril had direct effect on hematocrit level in hemodialysis patients. Patients who were not taking enalapril or stopped taking enalpril at least 4 months before the study, then started taking enalapril for 4 months, their Hct levels were decreased significantly. For patients who were taking enalapril for at least 4 months, then, enalapril was stopped for 4 months, their Hct levels were increased significantly. The decrement in Hct levels caused by enalapril was more prominent in the patients who were not treated with erythropoietin as compared to those who were taken erythropoietin.

2. Higher dosage of enalpril up to 10 mg/day, caused higher effect on the decrement in Hct levels. Further increment in the dosage of enalapril did not show further increment in the percentage of reduction in Hct levels.

3. Erythropoietin is a significant factor related to Hct synthesis. The effect of enalapril on Hct levels was therefore most apparent in the group of patients who were not treated with erythropoietin and consumed high dose of enalapril. However, among patients who were treated with erythropoietin, further categorized patients into two subgroups consuming $\geq$80 units/kg/week and <80 units/kg/week of erythropoietin did not show any significantly different between the two subgroups. Patients who require treatment with high dosage of erythropoietin (even when enalapril has not been given) are usually lost higher amount of Hct along their normal process of clinical treatment conditions without the effect of enalapril). This might interfere with the observation of the effect of enalapril.

4. Only 13 patients were on the same dosage of enalapril and erythropoietin for 12 months, these 13 patients were therefore observed for the duration of effect of enalapril on Hct level. The effect of enalapril on the decrement in Hct level was significant at the first 4 months, the decrement was slightly further at the second 4 months (8 months), then, at the third 4 months (12 months), the decrement did not move further, in contrary, the Hct level was slightly increased. This might due to the saturation of the effect of enalapril or the process become tolerant or the regulator process of the body might take place mostly within 8 months.

   For the rest of the patients, those who were not on erythropoietin were mostly discontinued from observation after the first or second 4 months due to requirement for addition of erythropoietin and/or packed red cell, while for those who were on erythropoietin were mostly discontinued from observation after the first or second 4 months due to changing in enalapril dosage.

5. Sex, age, duration of hemodialysis and frequency of dialysis did not affect the effect of enalapril on Hct levels of the patients.

This study had several favorable points. For instance, the number of subjects recruited into this study was higher than most previous studies, the monitoring time was longer, the study design was a before and after treatment with enalapril pattern, so the patients became self controlled which could help eliminated several confounding factors. The study compared the effects of enalapril in patients who were and were not treated with erythropoietin, since erythropoietin is a strongly confounding factor of Hct level, the effect of enalapril could then be clearly concluded in patients who were not treated with erythropoietin. In addition, this study also categorized patients according to enalapril dosages and monitoring

on the constant conditions of the patients for 12 months period, these parts of information had never been reported.

The present study has several limitations. First, this study was a retrospective study. Second, enalapril might not be the only factor that caused decreasing in Hct levels since several other confounding factors such as serum parathyroid hormone, serum ferritin that often had been monitored and controlled in most studies had not been recorded and controlled in our study. Third, the effect of erythropoietin and packed red cell might be prolonged for more than 4 months, therefore, the dosage of erythropoietin and packed red cell should be kept stable for at least 8 months to make sure that these confounding factors will not interfere with the results. Fourth, the number of patients recruited were too small especially for categorized into different subgroups with different dosages of enalapril and/or erythropoietin.

Further studies using prospective design in higher number of patients and better controls of all the confounding factors are desired before any definite conclusion could be made.

REFERENCES

1. Albitar, S., Genin, R., Chong, M. F., Serveaux, M. O., and Bourgeon, B. High dose enalapril impairs the response to erythropoietin treatment in haemodialysis patients. Nephrol Dial Transplant 13 (1998): 1206-1210.
2. Erturk, S., and others. The impact of withdrawing ACE inhibitors on erythropoietin responsiveness and left ventricular hypertrophy in haemodialysis patients. Nephrol Dial Transplant 14 (1999): 1912-1916.
3. Macdougall, I. C. The role of ACE inhibitors and angiotensin II receptor blockers in the response to epoetin. Nephrol Dial Transplant 4 (1999): 1836-1841.
4. Hirakata, H., Onoyama, K., Hori, K., and Fujishima, M. Participation of the rennin-angiotensin system in the captopril-induced worsening of anemia in chronic hemodialysis patients. Clin Nephrol 26 (1986): 27-32.
5. Dhondt, A. W., Vanholder, R. C. and Ringoir, S. M. G. Angiotensin-converting enzyme inhibitors and higher erythropoietin requirement in chronic haemodialysis patients. Nephrol Dial Transplant 10 (1995): 2107-2109.
6. Matsumura, M., Nomura, H., Koni, I., and Mabuchi, H. Angiotensin-converting enzyme inhibitors are associated with the need for increased recombinant human erythropoietin maintenance doses in hemodialysis patients. Nephron 77 (1997): 164-168.
7. Hess, E., Sperchneider, H., and Stein, G. Do ACE inhibitors influence the dose of human recombinant erythropoietin in dialysis patients? Nephrol Dial Transplant [letters] (1996): 749-750.
8. Erturk, S., Ates, K., Duman, N., Karatan, O., Erbay, B., and Ertug, E. Unresponsiveness to recombinant human erythropoietin in haemodialysis patients: possible implications of angiotensin-converting enzyme inhibitors. Nephrol Dial Transplant [letters] (1996): 396-397.

9. Walter, J. Does captopril decrease the effect of human recombinant erythropoietin in haemodialysis patients? Nephrol Dial Transplant [letters] 8 (1993): 1428-1431.

10. Charytan, C., Rumyantzev, A. G., Wang, Y. F., Schwenk, M. H., and Spinowitz, B. S. Effect of angiotensin-converting enzyme inhibitors on response to erythropoietin therapy in chronic dialysis patients. Am J Nephrol 18 (1998): 498-503.

11. Abu-Alfa, A. K., Cruz, D., Perazella, M. A., Mahnensmith, R. L., Simon, D., and Bia, M. J. ACE inhibitors do not induce recombinant human erythropoeitin resistance in hemodialysis patients. Am J Kidney Dis 35 (2000): 1076-1082.

12. Hayashi, K., Hasegawa, K., and Kobayashi, S. Effects of angiotensin-converting enzyme inhibitors on the treatment of anemia with erythropoietin. Kidney International 60 (2001): 1910-1916.

13. Cruz, D. N., Perazella, M. A., Abu-Alfa, A. K., and Mahnensmith, R. L. Angiotensin-converting enzyme inhibitor therapy in chronic hemodialysis patients: any evidence of erythropoietin resistance? Am J Kidney Dis 28 (1996): 535-540.

14. Sanchez, J. A., and Tomero. ACE inhibitors do not decrease rHuEpo response in patients with end-stage renal failure. Nephrol Dial Transplant [letters] (1995): 1476-1477.

15. Herfindal, E. T., and Gourley, D. R. Textbook of therapeutics drug and disease management. 7th ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

16. Lappin, T. The cellular biology of erythropoietin receptors. The oncologist 8 (2003): 15-18.

17. Santi, D. V., and Ries, C. A. Agents used in anemias; hematopoietic growth factors. In Katzung, B. G. (ed.), Basic & clinical pharmacology, pp 533-546, 1998.

18. Litvak, K. AHFS drug information. American society of health system pharmacists, 1999.

19. Krauss, A. G., and Hak, L. J. Chronic renal disease. In Herfindal, E. T., and Gourley, D. R. (ed.), Textbook of therapeutics drug and disease management, 447-482. Philadelphia: Lippincott Williams & Wilkins, 2000.

20. Bauer, J. H., and Reams, G. P. Antihypertensive drugs. In Brenner, B. M. (ed.), The kidney, pp 2253-2297. Philadelphia: W.B. Saunders, 2000.

21. Litvak, K. AHFS drug information. American society of health system pharmacists, 1999.

22. Reid, I. A. Vasoactive peptides. In Katzung, B. G. (ed.), Basic & clinical pharmacology, pp 287-303, 1998.

23. Lacy, C. F., Armstrong, L. L., Goldman, M. P., and Lance, L. L. Drug information handbook. 12th ed. Ohio: Lexi Comp, 2004.

24. ศรีสมร รัตนจินดา. ผลของยา ACEI และ AIIB ที่มีต่อขนาดยา erythropoietin ในผู้ป่วยโรคไตวายเรื้อรังที่ได้รับการฟอกเลือด ณ โรงพยาบาลราชวิถี. รายงานปัญหาพิเศษทางเภสัชกรรม. โครงการจัดตั้งภาควิชาเภสัชกรรมคลินิก คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. 2546. [เอกสารไม่ตีพิมพ์]

25. Cole, J., and others. Lack of angiotensin II-facilitated erythropoiesis causes anemia in angiotensin-converting enzyme-deficient mice. J Clin Invest 106 (2000): 1391-1398.

26. Naito, M., Kawashima, A., Akiba, T., Takanashi, M., and Nihei, H. Effects of an angiotensin II receptor antagonist and angiotensin-converting enzyme inhibitors on burst forming units-erythroid in chronic hemodialysis patients. Am J Nephrol 23 (2003): 287-293.

27. Azizi, M., Rousseau, A., and Ezan, E. Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator N-Acetyl-Seryl-Aspartyl-Lysyl-Proline. J Clin Invest 97 (1996): 839-844.

28. Constantinescu, C. S., Goodman, D. B. P., and Ventura, E. S. Captopril and lisinopril suppress production of interleukin-12 by human peripheral blood mononuclear cells. Immunol Lett 62 (1998): 25-31.

29. St Peter, W. L., Schoolwerth, A. C., McGowan, T., and McClellan, W. M. Chronic kidney disease: issues and establishing programs and clinics for improved patient outcomes. Am J Kidney Dis 41 (2003): 903-924.

30. อุดม ไกรฤทธิชัย. หลักการใช้ Erythropoietin ในผู้ป่วยโรคไตวายเรื้อรัง:ประเสริฐ ธนกิจจารุ, วสันต์ สุเมธกุล, เกรียง ตั้งสง่า, บรรณนาธิการ. DRUGS AND THE KIDNEY, หน้า 266-276. กรุงเทพมหานคร: สมาคมโรคไตแห่งประเทศไทย, 2538.

31. National Kidney Foundation: K/DOQI Clinical Practice Guidelines for theTreatment of Anemia of Chronic Kidney Disease: Update 2000. Am J Kidney Dis 14 (2001): S182-238 (suppl 1).

32. ดุสิต ล้ำเลิศกุล, วุฒิเดช โอภาศเจริญสุข. Angiotensin converting enzyme inhibitors and renal diseases. ใน: ประเสริฐ ธนกิจจารุ, วสันต์ สุเมธกุล, เกรียง ตั้งสง่า, บรรณนาธิการ. DRUGS AND THE KIDNEY, หน้า 63-82. กรุงเทพมหานคร: สมาคมโรคไตแห่งประเทศไทย, 2538.

33. อรุณี หาญทวีพันธุ์. ยาลดความดันโลหิต. กรุงเทพมหานคร: เมดอินโฟ จี.ดี., 2542.

34. เกรียง ตั้งสง่า, สมชาย เอี่ยมอ่อง, บรรณาธิการ. Hemodialysis. กรุงเทพมหานคร: Text and Journal Publication, 2542.

35. Hillman, R. S., and Ault, K. A. Hematology in clinical practice: a guide to diagnosis and management. 2nd ed. New York: McGraw-Hill, 1998.

36. Fishbane, S. Anemia treatment in chronic renal insufficiency. Seminars in Nephrology 22 (2002): 474-478.

37. Efrati, S., and others. ACE inhibitors and survival of hemodialysis patients. Am J Kidney Dis 40 (2002): 1023-1029.

38. Meur, Y. L., and others. Plasma levels and metabolism of AcSDKP in patients with chronic renal failure : relationship with erythropoietin requirements. Am J Kidney Dis 38 (2001): 510-517.

39. Quesenberry, P. J. Hemopoietic stem cells, progenitor cells, and cytokines. In Beutler, E. B., Lichtman, M. A., Coller, B. S., and Kipps, T. J. (eds.), Hematology, pp.211-228. New-York: McGraw-Hill, 1995.

40. Erslev, A. J. In Beutler, E. B., Lichtman, M. A., Coller, B. S., and Kipps, T. J. (eds.), Hematology, pp.456-462. New-York: McGraw-Hill, 1995.

41. Macdougall, I. C. Poor response to erythropoietin: practical guidelines on investigation and management. Nephrol Dial Transplant 10 (1995): 607-614.

42. Holmes, H. N. Handbook of diagnostic tests. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2003.

43. เล็ก รุ่งเรืองยิ่งยศ. การตรวจทางห้องปฏิบัติการ. ใน: เฉลิมศรี ภุมมางกูร, กฤตติกา ตัญญะแสนสุข, บรรณาธิการ. โอสถกรรมศาสตร์, หน้า 105-142. กรุงเทพมหานคร: นิวไทยมิตร, 2543.

APPENDICES

Appendix A

PATIENT GENERAL DATA FORM

ข้อมูลทั่วไปของผู้ป่วย

1. ชื่อผู้ป่วย..... นามสกุล..... วันเกิด(วัน/เดือน/ปี).....
2. เพศ ☐ ชาย ☐ หญิง
3. วันเดือนปี ที่เริ่มรักษาโรคไตวายเรื้อรังครั้งแรก .....
4. วิธีการรักษาทดแทนไตครั้งแรก ☐ Hemodialysis ☐ Peritoneal dialysi ☐ Kidney transplant
5. สถาบันที่ให้การรักษาทดแทนไตครั้งแรก ☐ ที่สถาบันนี้ ☐ ที่สถาบันอื่น.....
6. สาเหตุของโรคไตวายเรื้อรัง

   ☐ Glomerulonephritis : Biopsy-proven (มีผล kidney biopsy)

   ○ Focal Segmental Glomerulonephritis ○ Membranous Nephropathy

   ○ Membranous Glomerulonephritis ○ IgA Nepropathy

   ○ Mesangial proliferative IgM Nephropathy ○ Crecentric Glomerulonephritis (RPGN)

   ☐ Glomerulonephritis : Presumed (no biopsy) ☐ Diabetic Nehropathy ☐ Hypertension

   ☐ Cystic Kidney Disease ☐ Analgesic Nephropathy ☐ Alport's Syndrome

   ☐ Obstructive Nephropathy ☐ Gouty/uric acid Nephropathy ☐ Lupus Nephritis

   ☐ Allograft Dysfunction ☐ Other causes(specify)……. .....☐ Unknown
7. Comorbid condition (อาการหรือการวินิจฉัยภายใน 10 ปี)

   .....

   .....

   .....
8. ความสูง (cm) ……..

ข้อมูลการรักษาด้วยการฟอกเลือด

9. เวลาเฉลี่ยในการฟอกเลือดต่อครั้ง (ชม. : นาที) ..... ชม. : ..... นาที
10. ความถี่ของการฟอกเลือดต่อสัปดาห์ ☐ 2 ครั้ง ☐ 3 ครั้ง
11. น้ำหนัก (dry weight) ขณะที่ได้รับยา enalapril (kg) …….
12. Vascular access ที่ใช้ ☐ AV fistular (shunt) ☐ AV graft

    ☐ Permanent catheter ☐ Temporary catheter

Appendix B

BLOOD CHEMISTRY SUMMARY SHEET

Date		Bs	BUN	Cr	Uric	Na	K	Cl	CO_3	Ca	P	Alb	Glob	Chol	TG	HBsAg	HBsAb	AntiHCV	AST	ALT	Semen Ferritin	TSAT	TIBC	Serum iron
	Pre																							
	Post																							
	Pre																							
	Post																							
	Pre																							
	Post																							
	Pre																							
	Post																							
	Pre																							
	Post																							

Appendix C

MONTHLY DATA COLLECTING FORM

Pt. No.	Dose(mg/d) Enalapril	Month 1								Month 2								Month 3								Month 4							
		E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4
	0																																
	5																																
	10																																
	20																																
	40																																
	0																																
	Dose(mg/d) Enalapril	Month 5								Month 6								Month 7								Month 8							
		E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4
	0																																
	5																																
	10																																
	20																																
	40																																
	0																																
	Dose(mg/d) Enalapril	Month 9								Month 10								Month 11								Month 12							
		E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4	E_1	H_1	E_2	H_2	E_3	H_3	E_4	H_4
	0																																
	5																																
	10																																
	20																																
	40																																
	0																																

Note: H refer to Hct levels

E refer to Erythropoietin dosage

Appendix D

PATIENT'S MEDICATION PROFILE

Enalapril, Iron injection

Date	Medication	Dosage/Administration	Off date

APPENDIX E

Demographic data of the individual subjects (patients who had not taken enalapril then start taking enalapril for 4, 8 and 12 months; n=48)

Code	Name	Sex	Age (years)	Dry weight (kg)	Height (cm)
A01	sommari	female	38	33.5	145.00
A02	wichaitoa	male	61	54.5	157.96
A03	somchai	male	60	69	165.00
A04	weerapol	male	32	67	165.00
A05	sakti	male	33	60.5	170.00
A06	kao	male	43	69.5	168.00
A07	somboon	male	58	43	158.00
A08	surin	male	31	45.5	150.00
A09	panya	female	41	50.5	150.00
A10	prasarn	male	41	57	162.00
A11	kamolphon	female	55	71.5	150.00
A12	prasuthep	male	53	47.5	165.00
A13	tanya	male	36	50.3	165.00
A14	kasame	male	45	59	170.00
A15	jaturong	male	34	78	195.00
A16	sompong	male	42	55	170.00
A17	narongsak	male	25	48.5	158.00
A18	pisitsanti	male	45	52	162.00
A19	suthepsuk	male	52	66.5	165.00
A20	nikorn	male	35	41	157.96
A21	panjarot	female	43	53.5	145.00
A22	chanon	male	22	50	157.96
A23	sutham	male	28	55	167.00
A24	surapon	male	47	65.8	163.00

Demographic data of the individual subjects (patients who had not taken enalapril then start taking enalapril for 4, 8 and 12 months; n=48) (cont.)

Code	Name	Sex	Age (years)	Dry weight (kg)	Height (cm)
A25	sompong	male	41	55.5	170.00
A26	subil	male	54	62	160.00
A27	montri	male	27	48	155.00
A28	pasin	male	52	52	161.00
A29	marasri	female	51	60	160.00
A30	satit	male	47	55.5	160.00
A31	janpeng	male	45	52	170.00
A32	nidarat	female	38	45	154.00
A33	banjong	female	43	43	157.96
A34	kraisorn	male	42	62.5	162.00
A35	somboon	female	32	38	157.96
A36	opart	male	28	55	170.00
A37	wichai	male	39	54.5	157.96
A38	sumreng	male	35	51.5	168.00
A39	phonsiri	female	48	47.5	159.00
A40	suchada	female	49	46.5	153.00
A41	wiparat	female	44	46	149.00
A42	montha	female	56	45	157.96
A43	rattana	female	31	44	145.00
A44	phontong	female	40	44	155.00
A45	somsakpan	male	35	43	152.00
A46	phontip	female	25	40.5	148.00
A47	piliarat	female	41	39.5	160.00
A48	somkid	female	24	39	157.96

APPENDIX F

Demographic data of the individual subjects (patients who had taken enalapril then stop taking enalapril for 4 months; n=9)

Code	Name	Sex	Age (years)	Dry weight (kg)	Height (cm)
B01	pisitjia	male	30	54.5	160.00
B02	sompop	male	28	58	157.96
B03	jintana	female	63	42	150.00
B04	chayakorn	male	44	53.5	157.96
B05	paiboon	female	48	95	160.00
B06	pranprai	male	32	45.5	167.00
B07	maliwan	female	62	40	148.00
B08	chutima	female	49	47.5	162.00
B09	siripan	female	33	42	157.96

APPENDIX G

Demographic data of the individual subjects (patients who had not taken enalapril then start taking enalapril for 4, 8 and 12 months; n=48)

Code	Cause of chronic kidney disease*	Comorbid condition**	Duration of hemodialysis (years)	Frequency of dialysis (times/wk)
A01	7	8	7	3
A02	18	6	9	2
A03	8	11	7	3
A04	7	8	6	3
A05	7	3	3	3
A06	7	3	4	2
A07	7	3	4	2
A08	18	3	2	2
A09	18	3	1	2
A10	7	6	11	3
A11	8	13	4	2
A12	16	3	10	2
A13	9	3	4	3
A14	8	10	9	3
A15	7	3	2	3
A16	18	3	6	3
A17	1	3	1	2
A18	7	3	1	3
A19	9	3	3	3
A20	5	16	1	2
A21	7	3	9	2
A22	7	16	2	2
A23	18	2	2	3
A24	8	13	8	3

Demographic data of the individual subjects (patients who had not taken enalapril then start taking enalapril for 4, 8 and 12 months; n=48) (cont.)

Code	Cause of chronic kidney disease*	Comorbid condition**	Duration of hemodialysis (years)	Frequency of dialysis (times/wk)
A25	9	3	2	2
A26	10	16	12	2
A27	9	8	4	2
A28	9	6	1	3
A29	9	12	7	3
A30	7	8	14	3
A31	18	8	6	2
A32	4	3	4	2
A33	7	3	2	2
A34	6	3	5	3
A35	9	3	4	2
A36	18	3	3	3
A37	7	15	2	3
A38	13	3	3	2
A39	18	3	7	2
A40	9	3	2	3
A41	7	3	6	2
A42	9	3	2	3
A43	7	3	5	2
A44	18	3	3	3
A45	7	3	5	2
A46	15	3	5	2
A47	7	3	8	3
A48	7	15	1	2

APPENDIX H

Demographic data of the individual subjects (patients who had taken enalapril then stop taking enalapril for 4 months; n=9)

Code	Cause of chronic kidney disease*	Comorbid condition**	Duration of hemodialysis (years)	Frequency of dialysis (times/wk)
B01	5	3	1	3
B02	7	16	2	2
B03	13	3	4	2
B04	7	3	15	3
B05	8	11	9	3
B06	13	3	4	3
B07	18	8	4	3
B08	13	8	7	3
B09	19	16	5	3

* Causes of chronic kidney disease	** Comorbid condition
1-Focal segmental glomerulonephritis (FSGN) 2-Membranous nephropathy 3-MPGN 4-IgA nephropathy 5-Mesangial proliferative IgM nephropathy 6-Crecentric glomerulonephritis (RPGN) 7-Glomerulonephritis (no biopsy) 8-Diabetic nephropathy 9-Hypertension 10-Cystic kidney disease 11-Analgesic nephropathy 12-Alport's syndrome 13-Obstructive nephropathy, CTIN 14-Gouty/uric acid nephropathy 15-Lupus nephritis 16-Allograft dysfunction 17-Other causes 18-Unknown	1-AMI or UA 2-CHF 3-HTN 4-DM 5-AMI or UA and CHF 6-AMI or UA and HTN 7-AMI or UA and DM 8-CHF and HTN 9-CHF and DM 10-HTN and DM 11-AMI or UA and CHF and HTN and DM 12-AMI or UA and CHF and HTN 13-AMI or UA and HTN and DM 14-AMI or UA and CHF and DM 15-none 16-no data

APPENDIX I

Laboratory data of the individual subjects before taking enalapril

Code	BUN	Cr	Uric	Na	K	Cl	CO_3	Ca	PO_4	Alb	AST	ALT	PTH	Ferritin	TIBC	Iron
A01	44	8.6	5.9	140	4.4	98	24	10.3	2.9	4.5	-	-	-	-	25.08	-
A02	63.1	12.2	9.1	142	4	102	21.1	9.5	1.9	4	-	-	93.63	-	-	-
A03	56	13.6	7	139	4.9	103	2.5	9.9	5.5	-	-	-	55.72	-	-	-
A04	57	12.6	-	141	4.2	101	22	9.7	5.3	-	-	-	-	-	-	-
A05	63	5.9	9.4	139	3.9	99	24	9.4	3.5	5	-	-	411.90	810.40	271.00	84.00
A06	89	19.5	11.7	140	5.2	99	20	9.8	6.8	4.4	-	-	-	-	-	-
A07	51	13.7	5.4	141	4.5	106	27	7.9	3.1	3.9	-	-	227.20	-	-	-
A08	101	24.2	10	142	6.2	108	13	8.3	11	4	-	-	-	-	-	-
A09	62	9.5	5.3	143	5.2	103	25	8.6	3.8	3.3	-	-	-	-	-	-
A10	69	13	5	143	5.2	100	30	10.4	5.2	4.1	-	-	-	-	-	-
A11	53.7	10	9.7	135	4.3	98	24.9	9.4	6	4.2	12	10	370.60	-	-	-
A12	60	10.1	8.3	142	5.4	102	26	8.9	3.7	3.4	-	-	-	-	-	-
A13	64	14.8	6.4	142	3.7	103	28	10.1	4.5	4.2	10	6	-	-	-	-
A14	45	6.6	5.2	142	4.4	102	30	8.6	4.1	3.8	45	41	-	-	-	-
A15	71	12.1	6.9	143	4.9	102	23	10.2	6.9	4.2	-	-	-	-	-	-
A16	31	7.3	4.3	132	4.7	93	30	7.7	6.1	4.6	-	-	-	-	-	-
A17	108	16.6	9.9	138	4.6	99	20	8.3	6.1	4.4	-	-	-	-	-	-
A18	55	15	7.6	138	4.4	96	19	9.6	5.4	3.8	-	-	-	-	-	-
A19	56	10.7	4.7	144	5.7	100	27	7.6	5.6	4.1	-	-	-	-	-	-
A20	34	6.3	4.5	141	3.2	103	25	10	4.9	4	19	22	16.19	-	-	-
A21	77	11.8	8	140	4.1	101	18	10.6	6.4	4	10	10	-	-	-	-
A22	67.6	14.6	8.2	148	4.6	102	23	8	6.4	4.1	13	13	289.50	887.00	206.00	92.00
A23	73	11.2	9.5	141	3.6	107	24	9.3	4	3.6	21	50	-	-	-	-
A24	86	10.7	8.4	145	5.9	101	16	11.6	7.8	4	14	9	-	-	-	-
A25	81	14.5	8.4	143	5.2	109	17	7.9	5.5	3.7	-	-	-	-	-	-
A26	61.7	10.5	8.02	136	4.5	96	27.9	8	4.8	4	-	-	-	-	-	-
A27	30	7.5	-	140	4.4	101	28	9.2	4.8	3.9	41	33	647.10	-	-	-
A28	-	11	8.3	138	4.8	103	20	9	5.9	4.3	-	-	-	140.50	138.00	55.00
A29	65	9.4	8.7	143	4.5	102	27	9.8	4	4.3	-	-	-	-	-	-
A30	38	8.7	4.3	142	4.4	92	30.3	10.3	4	4.6	-	-	558.70	-	-	-
A31	63	14	-	139	4.5	102	24.9	2.64	1.43	-	-	-	78.68	-	-	-
A32	68.7	16	9.6	141	5.9	100	23.2	10	9.1	4.1	-	-	374.60	-	-	-
A33	37	7.6	5.8	146	3.5	105	30	9.5	5.5	-	-	-	-	-	-	-
A34	61	15	6	134	4.2	100	22	9.7	3.6	4.1	-	-	14.36	708.80	32.20	49.00
A35	95.8	16	10.4	135	4.2	98	22.8	10.2	2.8	3.7	11	7	22.78	-	-	-
A36	52	11.4	5.4	142	4.1	101	25	9.4	3.8	4.6	-	-	809.50	984.40	256.00	60.00
A37	38	10.1	5.5	143	4.3	100	28	10.3	2.9	4.3	-	-	-	-	-	-
A38	80	16.7	6.2	142	4.9	107	17.6	9.2	5	-	18	10	189.90	-	-	-
A39	59	10	-	136	4.6	96	24	11.2	7.4	3.8	-	-	9.25	-	-	-
A40	82	13.9	7.1	143	4	104	18.9	10.5	4.3	-	-	-	50.38	-	-	-
A41	53	9.9	8.1	141	4.1	100	28	9.7	3.6	4.2	-	-	-	351.00	-	-
A42	31	5.5	3.4	142	4.4	103	31	13.3	3.3	4.2	41	41	5.58	-	-	-
A43	104	17.1	8.8	143	5.2	102	28	10	5.8	-	19	13	-	-	-	-
A44	52	12.6	-	142	4.2	101	25	9.9	5.5	4.6	-	-	1361.00		-	-
A45	66	10.9	9.6	145	5.6	107	21	9.6	6.3	4.1	-	-	-	-	-	-
A46	60	11	7.9	137	5.2	98	26	10.4	6.1	4.2	-	-	141.50	-	-	-
A47	95.1	9.9	8.3	137	5.3	100	22	10.2	3.6	4.4	10	11	232.20	-	-	-
A48	81	11.8	7.4	139	4.2	92	30	9.6	4.8	4.5	-	-	-	483.70	260.00	72.00

Laboratory data of the individual subjects before taking enalapril (cont.)

Code	BUN	Cr	Uric	Na	K	Cl	CO_3	Ca	PO_4	Alb	AST	ALT	PTH	Ferritin	TIBC	Iron
B01	30	7.6	2.9	139	3.6	96	22	12	4.1	5	-	-	-	-	-	-
B02	63	15.6	5.8	142	4.7	102	27	8.7	7.7	4	-	-	-	-	-	-
B03	48	6.9	-	143	4.2	98	23	-	1.6	4.4	-	-	635.40	-	-	-
B04	69.1	13	9.8	142	4.5	100	24.8	10.5	5.3	4.4	9	6	111.50	-	-	-
B05	51	9.7	9.6	140	4.5	102	23	11.5	4.9	4.1	-	-	290.00	-	-	-
B06	127	10.2	8.4	143	3.3	100	29	9.2	2.5	5.4	-	-	623.10	-	-	-
B07	30.1	7.6	5.1	139	4	104	27	9.5	2.7	4.7	-	-	-	-	-	-
B08	-	8.4	6.8	141	4.6	103	19	10.2	5	4.1	21	13	293.50	1058.00	-	-
B09	66.3	11.3	-	140	5.3	99	25.7	10	5.5	4.6	-	-	-	-	-	-

APPENDIX J

Laboratory data of the individual subjects after taking enalapril

Code	BUN	Cr	Uric	Na	K	Cl	CO_3	Ca	PO_4	Alb	AST	ALT	PTH	Ferritin	TIBC	Iron
A01	46	8.2	6.3	139	4.4	93	21	9.4	2.6	4.2	-	-	184.00	-	-	-
A02	-	8.6	6.5	142	4.2	105	18	8.7	3.1	4	27	23	548.00	82.10	346.00	13.00
A03	62	12.5	7.8	136	4.6	98	27	9.6	4.2	4.5	5	5	-	233.00	239.00	130.00
A04	52	10.5	6.6	144	4.5	106	26	7.6	2.4	4.4	17	18	139.00	-	-	-
A05	76	7.2	11.4	139	3.6	102	25.3	9.1	4.2	4.3	-	-	-	-	-	-
A06	-	10.8	7	140	5.7	102	24	10.4	6.6	4.7	16	15	458.80	-	-	-
A07	52	11	5.2	137	5.1	106	28	8.5	3.7	3.7	25	13	52.10	-	-	-
A08	-	8.3	-	146	5	108	28	9.1	6.4	-	-	-	1077.00	-	-	-
A09	107	12.1	8.5	138	6.3	102	19	8.4	4.8	3.7	-	-	-	-	-	-
A10	78	11.7	7	141	3.8	99	19.9	10.5	3.4	-	25	26	1003.00	-	-	-
A11	82	10.7	7.5	138	4	99	29	10.3	4.5	3.8	-	-	254.00	-	-	-
A12	82	9.8	9	137	5.6	119	21	9.4	4.7	3.9	16	11	-	-	-	-
A13	65	12.4	5.5	143	3.9	104	26	10.1	3.8	4.2	-	-	-	350.00	-	-
A14	40	5.4	4.7	141	4.2	108	21	9.1	4.5	3.7	-	-	-	-	-	-
A15	42	7.4	4.3	146	4.3	104	23	9.5	8.7	4	-	-	565.80	-	-	-
A16	57	11.3	6.4	136	5.1	98	22	8.2	4.5	4.2	10	10	-	-	-	-
A17	-	11.8	7.1	148	5.4	103	30	8.7	3.1	4.7	43	25	122.10	-	-	-
A18	85	16.3	9	137	4.1	98	23	9.2	4.5	3.8	9	12	-	-	-	-
A19	-	13.3	8.5	132	5.8	98	22.7	14.4	8.2	3.9	21	31	30.70	-	-	-
A20	49	9.2	-	140	5.6	-	24	7.6	7.8	2.8	-	-	-	-	-	-
A21	83	12.9	9.1	139	4.2	101	25	10.6	7.4	3.7	-	-	801.80	-	-	-
A22	71	14.5	-	144	5.4	106	16	9.1	5.8	4.1	4	13	-	-	-	-
A23	41	8.3	5.5	139	4.1	105	20	11	3.9	4.5	11	10	-	-	-	-
A24	76	10.1	6.7	145	5.2	105	24	11.7	6.4	4.2	-	-	-	-	-	-
A25	91	16	9.7	140	5.3	107	19	7.9	4.9	4	11	10	333.50	-	-	-
A26	49	11.2	7.62	135	4.1	102	24.2	8.2	5.1	3.8	-	-	-	-	-	-
A27	67	13	8.7	136	5.8	102	27	12.8	6.7	3.5	-	-	35.54	-	-	-
A28	66.9	11.7	7.6	143	4.2	97	23	2.49	2.2	4.3	-	-	338.00	-	247.00	74.00
A29	-	6.4	5	142	6.7	106	20	11.8	3.6	4.2	11	12	667.40	-	-	-
A30	-	-	8.6	138	3.3	94	25.9	2.4	0.66	4.7	-	-	470.40	-	-	-
A31	-	-	-	140	4.9	101	24.8	9.8	9.2	4.6	-	-	-	-	-	-
A32	55	7.2	9.5	139	4.8	100	26	9.1	7	4.1	-	-	588.70	-	-	-
A33	47	8.4	5.7	142	3.8	104	26	-	-	-	-	-	-	-	-	-
A34	44	11.4	-	137	5	102	22	9.4	4.7	4.3	-	-	-	733.00	193.10	71.00
A35	-	9.9	5.6	139	4.8	103	24	8.6	4.5	3.6	-	-	-	-	-	-
A36	64	10.6	6.6	142	4.6	100	27	9.1	4.8	4.5	-	-	-	705.80	288.00	92.00
A37	45	11.6	6.4	140	5.7	97	26	-	-	-	-	-	-	-	-	-
A38	71.6	18.6	9.6	146	4.7	99	26.6	9.6	5.3	3.7	-	-	-	-	-	-
A39	82	11.4	8.5	136	4.6	95	22	9.3	5.3	3.5	21	11	-	-	-	-
A40	62	10.1	4.5	136	4.9	103	24.2	10.7	4.2	-	-	-	-	-	-	-
A41	63	9.2	8.3	138	5	98	25	9	8.8	4.1	17	14	-	1610.00	338.00	89.00
A42	77	8.7	-	136	6.5	97	22	10.3	5.5	4.3	-	-	68.60	165.70	220.00	-
A43	92	17.1	9.1	142	5.4	99	27	10.7	6.2	3.7	-	-	-	-	-	-
A44	42	11.2	9.1	143	4	106	24	9.1	4.3	4.3	22	16	1029.00	-	-	-
A45	76	11.8	10.6	143	4.7	103	28	9.5	5.6	4.3	20	15	-	1400.00	-	-
A46	54	13.1	7.9	138	5.1	93	27	12.5	6.8	4.5	-	-	-	-	-	-
A47	50.3	9	6.6	139	4.3	101	20.7	9.2	2.3	4.1	-	-	-	-	-	-
A48	61	9.2	4.9	138	5.8	97	33	9.6	2.7	4	-	-	-	350.10	331.00	113.00

Laboratory data of the individual subjects after taking enalapril (cont.)

Code	BUN	Cr	Uric	Na	K	Cl	CO_3	Ca	PO_4	Alb	AST	ALT	PTH	Ferritin	TIBC	Iron
B01	38	9.5	4.9	142	4.9	95	29	9.4	3.9	3.7	58	125	-	-	-	-
B02	63	13	6.3	144	5	104	24	10.9	8.4	4.5	-	-	102.00	-	-	-
B03	45	7.9	-	143	3.8	98	26	10.2	2.5	4.5	-	-	-	-	-	-
B04	81.8	12.1	11	138	4.6	96	21	10	4.4	4.4	-	-	585.50	-	-	-
B05	53	9.2	8.4	139	4.5	100	24	10.8	5.7	4	-	-	330.10	373.00	301.00	122.00
B06	78	11.2	7	140	4.3	99	26	8.8	3.9	4.3	-	-	1263.00	-	-	-
B07	44	10.5	7.4	141	5.5	107	23.8	9.3	2.9	4	-	-	41.30	-	-	-
B08	85	11.2	10	135	4	98	15	9.9	6.6	4.2	-	-	-	220.50	-	-
B09	71.7	10.5	-	140	5.1	95	27.2	9.28	6	4.5	9	7	309.46	357.80	39.00	63.00

APPENDIX K

Hematocrit levels and percentage of change data of the individual subjects

Code	Dose EPO (unit/kg/wk)	Dose EPO (unit/wk)	Dose of enalapril (mg/day)	Hct levels every 4 months (%)				Percentage of change every 4 months (%)		
				Base line	4	8	12	4	8	12
A01	0	0	2.5	24	23	-	-	-4.17		
A02	0	0	5	27	26	-	-	-3.70		
A03	0	0	5	41	35	-	-	-14.63		
A04	0	0	5	26	25	30	32	-3.85	15.38	23.08
A05	0	0	5	30	28	28	-	-6.67	-6.67	
A06	0	0	5	42	33	-	-	-21.43		
A07	0	0	5	21	20	21	-	-4.76	0.00	
A08	0	0	5	24	23	25	26	-4.17	4.17	8.33
A09	0	0	5	21	19	18	17	-9.52	-14.29	-19.05
A10	0	0	5	31	28	-	-	-9.68		
A11	0	0	5	28	26	27	27	-7.14	-3.57	-3.57
A12	0	0	10	24	16	-	-	-33.33		
A13	0	0	10	33	18	-	-	-45.45		
A14	0	0	10	40	37	20	28	-7.50	-50.00	-30.00
A15	0	0	10	26	25	-	-	-3.85		
A16	0	0	10	28	23	23	-	-17.86	-17.86	
A17	0	0	10	21	16	-	-	-23.81		
A18	0	0	10	28	24	20	-	-14.29	-28.57	
A19	0	0	20	45	42	-	-	-6.67		
A20	0	0	20	20	21	-	-	5.00		
A21	0	0	20	29	25	30	31	-13.79	3.45	6.90
A22	0	0	40	21	18	-	-	-14.29		
A23	0	0	20	25	18	-	-	-28.00		
A24	0	0	40	28	28	33	35	0.00	17.86	25.00
A25	0	0	40	25	21	22	20	-16.00	-12.00	-20.00
A26	0	0	40	43	38	31	27	-11.63	-27.91	-37.21
A27	20.83	1000	10	30	28	26	-	-6.67	-13.33	
A28	28.85	1500	5	32	30	-	-	-6.25		
A29	33.33	2000	20	26	25	-	-	-3.85		
A30	36.04	2000	20	35	28	-	-	-20.00		
A31	38.46	2000	5	30	28	-	-	-6.67		
A32	44.44	2000	20	29	25	-	-	-13.79		
A33	46.51	2000	20	30	29	28	-	-3.33	-6.67	
A34	48	3000	40	31	30	27	29	-3.23	-12.90	0.00
A35	52.63	2000	5	24	20	21	-	-16.67	-12.50	
A36	72.73	4000	5	35	31	-	-	-11.43		
A37	73.39	4000	20	29	29	-	-	0.00		
A38	77.67	4000	5	31	31	29	33	0.00	-6.45	6.45
A39	84.21	4000	10	31	29	-	-	-6.45		
A40	86.02	4000	5	33	33	30	33	0.00	-9.09	0.00
A41	86.96	4000	5	25	21	-	-	-16.00		
A42	88.89	4000	5	34	37	-	-	8.82		
A43	90.91	4000	10	29	25	23	-	-13.79	-20.69	
A44	90.91	4000	40	33	31	32	-	-6.06	-3.03	
A45	93.02	4000	5	27	25	25	-	-7.41	-7.41	
A46	98.77	4000	20	34	35	-	-	2.94		
A47	101.27	4000	10	34	30	35	35	-11.76	2.94	2.94
A48	102.56	4000	20	40	30	-	-	-25.00		

Hematocrit levels and percentage of change data of the individual subjects (cont.)

Code	Dose EPO (unit/kg/wk)	Dose EPO (unit/wk)	Dose of enalapril (mg/day)	Hct levels every 4 months (%)				Percentage of change every 4 months (%)		
				Base line	4	8	12	4	8	12
B01	0	0	5	33	25	-	-	-24.24		
B02	0	0	5	32	32	-	-	0.00		
B03	0	0	10	25	24	-	-	-4.00		
B04	0	0	40	39	36	-	-	-7.69		
B05	42.11	4000	5	35	34	-	-	-2.86		
B06	43.96	2000	10	22	21	-	-	-4.55		
B07	50	2000	5	27	27	-	-	0.00		
B08	84.21	4000	5	36	33	-	-	-8.33		
B09	142.86	6000	20	34	30	-	-	-11.76		

BIOGRAPHY

Miss Srisamorn Rattanajinda was born on the 12th of February in 1976 at Pattani province. She graduated, in 1997, for Bachelor Degree of Science in Pharmacy from Faculty of Pharmaceutical Sciences, Chulalongkorn University. After that, she had been the pharmacist in Patoa hospital, Patoa district, Chumphon province until 2000. Then she moved to work at Chumphon hospital, Muang district, Chumphon province. In 2002, she had begun the graduated study at Chulalongkorn University for Master Degree of Science in Pharmacy, Program in Clinical Pharmacy, Department of Pharmacy, Faculty of Pharmaceutical Sciences.