The Thai Journal of Veterinary Medicine

Volume 41
Issue 2 *July, 2011*

Article 13

7-1-2011

Effects of Erythropoietin on Blood Pressure, Renal Function and Red Blood Cell Production in Dogs with Chronic Kidney Disease with and without Angiotensin Converting Enzyme Inhibitor

Sujittra Ritthikulprasert

Metinee Rodphol

Adthaphonl Nimrotham

Saikaew Sattayatham

Siripen Komolvanich

See next page for additional authors

Effects of Erythropoietin on Blood Pressure, Renal Function and Red Blood Cell Production in Dogs with Chronic Kidney Disease with and without Angiotensin Converting Enzyme Inhibitor

Authors

Sujittra Ritthikulprasert, Metinee Rodphol, Adthaphonl Nimrotham, Saikaew Sattayatham, Siripen Komolvanich, Monkon Trisiriroj, and Chollada Buranakarl

Original Article

Effects of Erythropoietin on Blood Pressure, Renal Function and Red Blood Cell Production in Dogs with Chronic Kidney Disease with and without Angiotensin Converting Enzyme Inhibitor

Sujittra Ritthikulprasert[1] Metinee Rodphol[1] Adthaphonl Nimrotham[1]
Saikaew Sattayatham[1] Siripen Komolvanich[1] Monkon Trisiriroj[2] Chollada Buranakarl[1*]

Abstract

Blood pressure, renal function, urinary protein excretion and red blood cell count were measured in 29 dogs with chronic kidney disease which received hormone recombinant human Erythropoietin (rHuEPO). The experiment was divided into 2 parts. Parts 1 comprised 3 groups of dogs. Group 1 dogs were studied prior to erythropoietin (EPO) injection (pre-EPO). Group 2 dogs received EPO 100 U/kg 2-3 times a week. Group 3 dogs received both EPO and angiotensin converting enzyme inhibitor which was enalapril 5 mg/kg/daily. The duration of medication was between 7 to 40 days. In part 2 of the experiment, all parameters were measured in the same dogs before and after the dogs received EPO alone or EPO with ACEI for 15 days. The results showed that dogs with CKD before EPO injection (group 1) had non regenerative anemia with elevated blood urea nitrogen and plasma creatinine concentrations. The blood pressure was within normal limit while the urinary excretion of protein, Na and K were enhanced. Group 2 and group 3 had no significant differences in all of these parameters except a significant increase in RBC production. Group 2 tended to have higher increase in RBC production more than group 3. In part 2 in which the study was performed in the same dogs, there were no changes in blood pressure and renal function. However, 15 days after EPO or EPO with ACEI, the significant increases in packed cell volume were found in dogs receiving EPO alone ($p<0.01$) and with ACEI ($p<0.05$) and the degree of EPO activated RBC production was greater in dogs receiving EPO alone ($p<0.05$). It is concluded that giving EPO either alone or with ACEI had no effect on blood pressure, renal function and urinary protein excretion in dogs with CKD, suggesting no angiotensin II involvement. However, by comparing with the same EPO intensity, dogs with CKD receiving ACEI required more EPO at the initial phase of treatment in order to yield the same increase in PCV.

Keywords: angiotensin converting enzyme inhibitor, blood pressure, dogs, erythropoietin, red blood cell production, renal function

[1] *Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University*
[2]*Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10330*
***Corresponding author E-mail:** bchollad@chula.ac.th*

บทคัดย่อ

ผลของฮอร์โมนอิริทรอพอยอิทิน ที่มีต่อความดันโลหิต การทำงานของไต และการสร้างเม็ดเลือดแดงในสุนัขที่มีโรคไตเรื้อรังที่ได้รับและไม่ได้รับยายับยั้งเอนไซม์เปลี่ยนแปลงแองจิโอเทนซิน

สุจิตรา ฤทธิกุลประเสริฐ [1] เมธินี รอดผล [1] อรรถพล นิมโรธรรม [1] ทรายแก้ว สัตยธรรม [1] ศิริเพ็ญ โกมลวานิช [1]
มนคน ตรีศิริโรจน์ [2] ชลลดา บูรณกาล [1]*

ทำการศึกษาความดันเลือด การทำงานของไต การขับทิ้งโปรตีนทางปัสสาวะ และการสร้างเม็ดเลือดแดงในสุนัข 29 ตัวที่เป็นโรคไตวายเรื้อรัง และได้รับฮอร์โมนอิริทรอพอยอิติน (EPO) ชนิด Recombinant human Erythropoietin (rHuEPO) การทดลองแบ่งเป็น 2 ส่วน ส่วนที่ 1 แบ่งสุนัขเป็น 3 กลุ่ม กลุ่มที่ 1 สุนัขเป็นไตวายเรื้อรังก่อนได้รับ EPO (pre-EPO) กลุ่มที่ 2 สุนัขได้รับ EPO ขนาด 100 ยูนิตต่อกก. 2-3 ครั้งต่อสัปดาห์ และกลุ่มที่ 3 สุนัขได้รับ EPO ร่วมกับยายับยั้งเอนไซม์เปลี่ยนแองจิโอเทนซิน (angiotensin converting enzyme inhibitor; ACEI) คือ enalapril maleate ขนาด 2.5 มก.ต่อกก. ทุกวัน ระยะเวลาที่ได้รับยาอยู่ระหว่าง 7-40 วัน ส่วนที่ 2 ทำการศึกษาในสุนัขตัวเดียวกันก่อนและหลังได้รับ EPO อย่างเดียว หรือ EPO ร่วมกับ ACEI เป็นเวลา 15-30 วัน ผลการศึกษาในส่วนที่ 1 พบว่า สุนัขในกลุ่มที่ 1 ก่อนได้รับ EPO มีภาวะเลือดจางแบบไม่มีการสร้างเม็ดเลือดแดงอ่อน และมีปริมาณยูเรียไนโตรเจนและครีอะตินีนในเลือดเพิ่มขึ้น ค่าความดันเลือดปกติในขณะที่การขับทิ้งโปรตีน โซเดียม และ โพแทสเซียมสูง ภายหลังให้ EPO อย่างเดียว (กลุ่มที่ 2) หรือให้ร่วมกับ ACEI (กลุ่มที่ 3) ไม่พบความแตกต่างของผลต่าง ๆ ยกเว้นการสร้างเม็ดเลือดแดง ที่เพิ่มขึ้น กลุ่มที่ 2 มีแนวโน้มของการเพิ่มขึ้นของเม็ดเลือดแดงมากกว่ากลุ่มที่ 3 ผลการศึกษาในส่วนที่ 2 ที่ศึกษาการเปลี่ยนแปลงในสุนัขตัวเดียวกันให้ผลเช่นเดียวกับส่วนที่ 1 โดยพบว่าความดันเลือดและการทำหน้าที่ของไต ไม่เปลี่ยนแปลง อย่างไรก็ตามเมื่อศึกษา 15 วันหลังได้รับยาพบว่าค่าเม็ดเลือดแดงอัดแน่นเพิ่มขึ้นอย่างมีนัยสำคัญในกลุ่มที่ได้รับ EPO อย่างเดียว ($p<0.01$) และกลุ่มที่ได้รับ EPO ร่วมกับ ACEI ($p<0.05$) การสร้างเม็ดเลือดแดงมีมากกว่าในกลุ่มที่ได้รับ EPO แต่เพียงอย่างเดียวเมื่อเทียบกับกลุ่มที่ได้รับ EPO ร่วมกับ ACEI ($p<0.05$) จากผลการทดลองสรุปได้ว่า การให้ EPO อย่างเดียวหรือร่วมกับ ACEI ไม่มีผลกับความดันเลือด การทำงานของไต และการขับทิ้งโปรตีนในปัสสาวะ ในสุนัขที่เป็นโรคไตวายเรื้อรังซึ่งบ่งบอกว่าผลของ EPO ไม่ได้ผ่านการทำงานของสารแองจิโอเทนซินทู (AII) อย่างไรก็ตามจากการเปรียบเทียบโดยใช้ความแรงของ EPO ที่เท่ากัน พบว่าสุนัขที่เป็นโรคไตวายเรื้อรังที่ได้รับ ACEI จะต้องการขนาดของ EPO มากกว่าในช่วงเริ่มต้นของการฉีดเพื่อให้ได้ค่าเม็ดเลือดแดงอัดแน่นเท่ากัน

คำสำคัญ: สารยับยั้งเอนไซม์เปลี่ยนแองจิโอเทนซิน ความดันโลหิต สุนัข อิริทรอพอยอิทิน การสร้างเม็ดเลือดแดง การทำงานของไต

[1] *ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*
[2] *คลินิกเฉพาะทางโรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330*
***ผู้รับผิดชอบบทความ E-mail:** bchollad@chula.ac.th*

Introduction

The nonregenerative normochromic normocytic anemia in chronic kidney disease is due to an inappropriate increase in EPO produced by epithelial cell in the kidney in response to anemia (King et al., 1992). The exogenous recombinant human erythropoietin (r-HuEPO), a hormone that stimulates red blood cell production has been widely used to correct anemia in both human and animals suffering from chronic kidney disease. Animals will show signs of clinical well-being, improved appetite and increased weight gain, alertness and cheerfulness after the hormone is used. The severity of anemia is associated with the severity of renal disease progression as shown by an inverse relationships between packed cell volume (PCV) and plasma creatinine concentration (King et al., 1992). However, r-HuEPO caused adverse effects in some animals such as systemic hypertension, seizure, iron deficiency as well as an anemia due to anti-r-HuEPO antibody (Cowgill et al., 1998).

The EPO induced hypertension along with increase in urinary albumin excretion were found in spontaneous hypertensive rat (Panzacchi et al., 1997). High blood pressure due to EPO may be associated with increased endothelin-1 (ET-1) (Slowinski et al.,

2002). A study of the effect of r-HuEPO in Munich-Wistar rats revealed that EPO could prevent anemia but worsen systemic and glomerular hypertension (Garcia et al., 1988). The EPO reduced glomerular filtration rate as well as renal blood flow leaving high filtration fraction in rats induced renal injury with gentamicin (Thongchai et al., 2008).

In dogs, increased blood pressure and/or proteinuria is associated with the risk of developing a uremic crisis or morbidity and mortality rates in dogs with chronic renal failure (Jacob et al., 2003, Jacob et al., 2005). Angiotensin converting enzyme inhibitor (ACEI) is one of the antihypertensive agents used in animals. A study in cats with reduced renal insufficiency showed the drug could reduce both systemic and glomerular capillary pressure (Brown et al., 2001). ACEI also reduced the urinary protein loss in renal disease in rats and human patients (Brooks et al., 1990; Keilani et al., 1993; Maschio et al., 1994; Buranakarl et al., 2003). Thus, ACEI has been prescribed in chronic renal failure animals frequently and may be prescribed in patients receiving chronic administration of EPO. Combination of EPO and ACEI has been observed regarding blood pressure, renal function and changes in packed cell volume. A study in hypertensive rat showed that hypertension along with increase in urinary albumin excretion after EPO injection could not be prevented by ACEI (Panzacchi et al., 1997). A report of using ACEI in patients with chronic renal failure showed impaired erythropoietin induced erythropoiesis (Qureshi et al., 2007; Rossert et al., 2007). However, negative results were also found (Cruz et al., 1996). Therefore, the aim of this study was to evaluate the changes in blood pressure, renal function and packed cell volume in dogs with chronic kidney disease receiving EPO alone and EPO in combination with ACEI.

Materials and Methods

The study was undertaken in 29 dogs (17 females and 12 males) with chronic kidney disease (CKD) were presented at The Small Animal Hospital, Chulalongkorn University, Bangkok, Thailand during year 2008-2009. CKD was diagnosed by prolonged medical treatment of azotemia, physical examination, urinalysis and radiographic imaging or ultrasonography. The pre-renal and post-renal azotemia were excluded from this study. The study was divided into 2 experiments. Experiment 1 was a study that was performed in 3 groups of dogs. The pre-EPO group (group 1) was defined by prior treatment of Epoitin alpha (Epokine®) with no ACEI (n=14). The EPO group (group 2) was defined by treatment with EPO for at least 7 days (n=7) while EPO+ACEI group (group 3) was defined by treatment of EPO along with ACEI (enalapril maleate) for at least 7 days (n=5). The dose of EPO was 100 U/kg and was given to the animals with a frequency between 2 to 3 times a week while the dose of ACEI was 5.0 mg/kg/day. Blood and urine samples including blood pressure measurement were performed on the day the dog were presented at the hospital along with the number of EPO injections and its protocol. Experiment 2 consisted of the CKD dogs in which the studies were repeated before and between 15 to 30 days after either EPO alone (n=5) or EPO with ACEI administrations (n=6). In this experiment, ACEI was given on the first day EPO was given except in one dog that ACEI was given a week later. The measurement of PCV in the second experiment was performed on day 0 and day 15 when EPO was administered in each individual dog with the same protocol leaving the number of dogs to be 4 for each group. The intensity of EPO administration (the average number of EPO injection) in both experiments was defined as the total numbers of EPO injection divided by the total day of EPO treatment.

The experimental and analytical procedures: All dogs were subjected to blood pressure measurement using oscillometric techniques (Fukuda, Tokyo, Japan). One milliliter of blood was collected into K-EDTA from each dog to perform the measurement of packed cell volume, number of red blood cell count and reticulocyte count in experiment 1 and only packed cell volume in experiment 2. Another one millilitre of blood collected in heparinized tube was centrifuged and plasma was separated for measurements of concentrations of creatinine, urea nitrogen and electrolytes (Na, K). The urine was collected by catheterization for measurement of concentrations of protein, creatinine and electrolytes (Na, K). Data from the urine were expressed as urinary protein creatinine ratio and fractional excretion of Na and K

Analytical procedures: The packed cell volume was measured by microcentrifugation method. The red blood cell count, were measured by automated analyzer (Mythic18, C2 Diagnostics, Montpellier, France). Reticulocyte count was performed by staining the reticulocyte with new methylene blue and counting under light microscope for 1000 cells. The % reticulocyte was expressed by correcting with the PCV. Blood urea nitrogen and both plasma and urine creatinine concentrations were measured by colorimetric methods using automate analyzer (BT2000, Biotecnica Instruments, S.p.A., Rome, Italy). Sodium and potassium in both plasma and urine were measured using flame photometry (Flame photometer, 41^{o}C, Ciba Corning Diagnostic Scientific Instruments, Halstead, UK). Urinary protein was quantified by turbidity by precipitation with sulphosalicylic acid.

Statistical analysis: All data are expressed as mean+SEM. The comparisons between groups of dogs in experiment 1 were performed using one way ANOVA on rank. In experiment 2, comparisons of data before and after treatment with either EPO alone or EPO with ACEI were performed using student paired-*t*-test. The unpaired t-test was used to analyze the data at the same period between EPO alone and EPO with ACEI. The significance was considered when *p*-values were less than 0.05. All statistical tests were performed using Sigma Stat program.

Results

Dogs with chronic kidney disease in experiment 1 consisted of 10 males and 16 females. The average age of dogs in group 1, 2 and 3 were

7.43±0.82, 6.71±0.58 and 6.2±0.58 years old,

Table 1 Blood pressure, plasma chemistries and urinary excretions of protein and electrolytes in 3 groups of dogs with CKD prior to EPO injection, EPO injection alone and EPO injection in combination with ACEI.

	Pre-EPO	EPO	EPO+ACEI
SAP (mmHg)	146.9±10.0 (13)	133.0±6.4 (7)	151.8±9.8 (4)
DAP (mmHg)	83.4±8.4 (13)	97.4±10.3 (7)	87.0±6.7 (4)
BUN (mg/dl)	154.6±15.1 (14)	152.3±28.3 (7)	135.2 ±11.0 (5)
PCr (mg/dl)	8.67±1.05 (14)	7.47±1.21 (7)	9.04±2.81 (5)
PNa (mEq/L)	138.7±1.85 (13)	142.6±3.08 (7)	140.4±1.7 (5)
PK (mEq/L)	5.08±0.26 (13)	5.03±0.49 (7)	5.72±0.65 (5)
UPC ratio	8.10±3.16 (12)	1.80±0.49 (7)	14.91±6.37 (5)
FENa (%)	9.93±2.01 (13)	10.10±3.17 (7)	6.98±2.05 (5)
FEK (%)	80.0±14.2 (13)	87.6±26.8 (7)	51.81±18.15 (5)

Data are presented as mean±SEM, numbers in parenthesis indicate number of dogs; SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, BUN: Blood urea nitrogen, PCr: Plasma creatinine concentration, PNa: Plasma sodium concentration, PK: Plasma potassium concentration, UPC ratio: Urinary protein creatinine ratio, FENa: Fractional excretion of sodium, FEK: Fractional excretion of potassium

Respectively, with a range between 1-13 years of age. All dogs were defined as CKD stage 2 to 4 categorized by IRIS (International Renal Interest System) based upon plasma creatinine concentration. The durations of EPO treatment in group 2 and 3 were 19.1±4.7 (ranging from 7 to 39) days and 19.4±3.8 (ranging from 10 to 30) days, respectively. The numbers of EPO injection in group 2 were between 3 to 12 with the average of 7.1 while in group 3 were between 5 to 12 with the average of 8.0. No significant differences were found between EPO administration expressed as the EPO intensity in group 2 and 3 (0.400±0.031 and 0.423±0.024, respectively). There were no significant differences among the 3 groups in blood pressure, plasma creatinine and BUN concentrations, plasma and fractional excretion of Na and K and the urinary protein creatinine ratio (Table 1).

Table 2 Packed cell volume and red blood cell parameters in 3 groups of dogs with CKD prior to EPO injection, EPO injection alone and EPO injection in combination with ACEI.

	Pre-EPO	EPO	EPO+ACEI
PCV (%)	20.46±1.82[a] (14)	31.50±2.27[b] (7)	29.4±2.44[b] (5)
RBC (x 10^3cells/mm^3)	2.38±0.39[a] (12)	4.57±0.53[b] (4)	4.0±0.71[b] (4)
Corrected reticulocyte count	0.027±0.007[a] (14)	0.459±0.144[b] (7)	0.359±0.113[b] (5)
MCV (fl^3)	94.19±10.82 (12)	70.61±10.78 (4)	77.09±5.96 (4)

Data are presented as mean±SEM; numbers in parenthesis indicate number of dogs; different superscripts within the same row differ significantly (p<0.05) using one way analysis of variance, PCV: Packed cell volume, RBC: Red blood cell count, MCV: Mean corpuscular volume

Table 2 shows the packed cell volume and red blood cell production in all 3 groups. The packed cell volume, number of red blood cell count and reticulocyte counts were significantly higher in group 2 and 3 compared with group 1 (p<0.05) while the MCV tended to be lower without any significant differences. The magnitudes of increase in PCV, RBC and reticulocyte count in group 2 were slightly higher than group 3 even though the EPO intensity was lower.

Table 3 Blood pressure, plasma chemistries, urinary excretions of protein and electrolytes and packed cell volume in dogs with CKD before and after EPO injection alone and EPO injection in combination with ACEI.

	Before EPO	After EPO	Before EPO+ACEI	After EPO+ACEI
SAP (mmHg)	144.0±10.8 (5)	132.4±13.1	143.0±18.4 (4)	146.8±13.7
DAP (mmHg)	77.8±5.8 (5)	83.2±5.5	78.0±11.6 (4)	84.8±8.9
BUN (mg/dl)	139.9±51.5 (5)	120.2±48.7	122.2±41.4 (5)	119.2±23.6
PCr (mg/dl)	7.35±2.49 (5)	5.16±2.08	7.72±2.07 (5)	8.34±3.19
PNa (mEq/L)	140.2±3.7 (5)	140.2±3.2	133.5±4.3 (4)	142.8±1.3
PK (mEq/L)	4.40±0.27 (5)	5.36±0.26*	5.13±0.26 (4)	6.28±0.54
UPC ratio	1.16±0.36 (5)	1.27±0.4	7.72±5.94 (5)	13.30±6.60 †
FENa (%)	14.05±5.77 (5)	9.37±4.68	5.99±1.98 (4)	6.31±3.07
FEK (%)	77.1±28.4 (5)	44.1±18.9	32.8±13.4 (4)	52.0±25.3
PCV (%)	19.75±0.95 (4)	38.75±3.95**	20.0±2.35 (4)	26.0±1.47* †

Data are presented as mean±SEM, numbers in parenthesis indicate number of dogs; *: p<0.05, **: p<0.01 compared with before treatment using student paired-t-test, †: p<0.05 compared with column 2 which received EPO alone using unpaired t-test, SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, BUN: Blood urea nitrogen, PCr: Plasma creatinine concentration, PNa: Plasma sodium concentration, PK: Plasma potassium concentration, UPC ratio: Urinary protein creatinine ratio, FENa: Fractional excretion of sodium, FEK: Fractional excretion of potassium, PCV: Packed cell volume

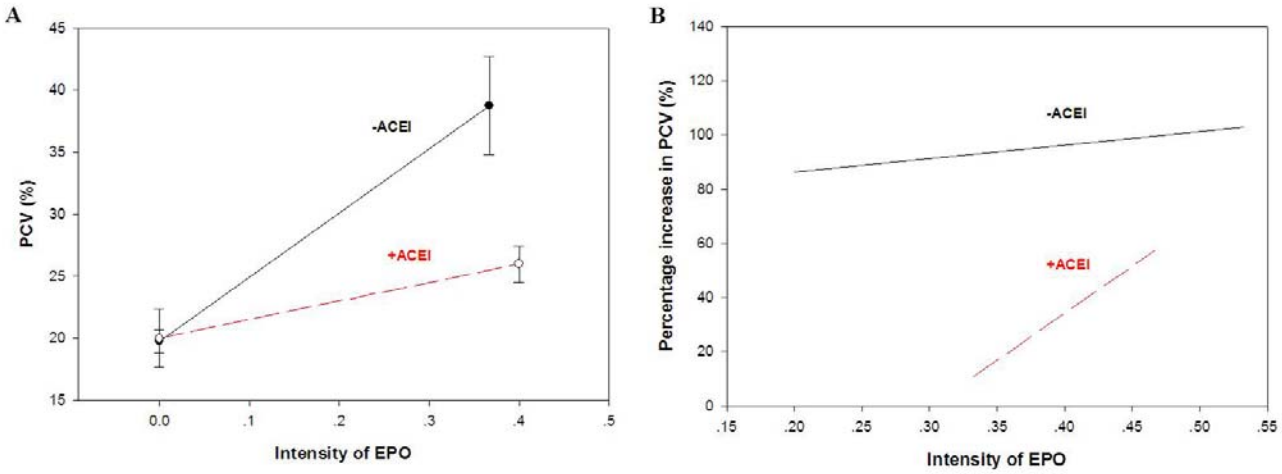

Figure 1 Packed cell volume (1A) and regression line estimating percentage increase in packed cell volume (1B) in dogs after 15 days of receipt of EPO alone without ACEI (solid line) and with ACEI (dash line).

In experiment 2, the number of EPO injection at 15 days of treatment in the groups receiving EPO alone and EPO in combination with ACEI were 5.8 and 7, respectively. The average age of the dogs receiving EPO alone and EPO in combination with ACEI was similar (5.75±1.7 and 5.75±0.48 years old, respectively). The intensities of EPO in the dogs receiving EPO alone and with ACEI were 0.333±0.07 and 0.386±0.025, respectively. The blood pressure, plasma chemistries except plasma K, urinary protein and electrolyte excretions were unchanged after the dogs received either EPO alone or EPO with ACEI (Table 3). Plasma K was significantly higher (p<0,05) after the dogs received EPO alone when compared with the value before EPO injection.

Subgroup of dogs in that PCV was measured had the average age of 7.20±1.96 years old in dogs receiving EPO alone and 6.33±0.49 years old in dogs receiving EPO in combination with ACEI. The intensities of EPO were 0.367±0.079 and 0.400±0.027 in the groups receiving EPO alone and EPO with ACEI, respectively. The PCV significantly increased after EPO was injected alone (p<0.01) or with ACEI (p<0.05) at 15 days of treatment (Table 3). However, the degree of PCV elevation in response to EPO without ACEI was higher than that in response to EPO with ACEI (p<0.05). By correcting the number and interval of EPO administration using the intensity value, it showed that the PCV of the dogs receiving EPO alone could response more dramatically than the dogs receiving EPO with ACEI (Fig 1A). When plotting the intensity of EPO and percentage changes in PCV in the first 15 days of administration, the intensity of EPO in dogs receiving EPO with ACEI would require more to produce the rate of PCV elevation compared with the dogs receiving EPO alone (Fig 1B).

Discussion

The blood urea nitrogen and the creatinine concentration of the dogs in experiment 1 were high and the average creatinine concentration was categorized as stage 4 CKD. These dogs had normal systolic and diastolic blood pressure; and high urinary excretion of protein, sodium and potassium which were similar to a previous report in CKD (Buranakarl et al., 2007). It was suggested that increased protein loss was directly related to the severity of renal impairment (Buranakarl et al., 2007). Dogs with high systolic blood pressure are more likely to develop the uremic crisis and to die than dogs without hypertension (Jacob et al., 2003). Moreover, a study of the same group demonstrated that the relative risks of developing uremic crisis and death were 3 times higher in dogs with UPC ratio equal or higher than 1.0 (Jacob et al., 2005). Moreover, the relative risk of adverse outcome was 1.5 times higher for every 1 unit increment of UPC (Jacob et al., 2005). Therefore, the dogs in group 1 and 3 may have higher risk for renal function deterioration. Moreover, the creatinine was slightly higher than the dogs in group 2. In group 2, every dog that received EPO from 3 to 13 injections showed no elevation in blood pressure. A study in human with hemodialysis showed an acute increase in blood pressure after 30 minutes of EPO injection (Miyashita et al., 2004). However, none of the predialysis patient became hypertensive. The increase in blood pressure corresponded to an increase in endothelin-1. However, in that study, the blood pressure was very little elevated (2 mmHg). The data also suggested that the blood pressure regulatory mechanism in response to EPO injection in hemodialysis patients reacted differently than in CRF patients without HD. More study in rats showed that EPO caused an increase in blood pressure in SHR, but not in WKY suggesting genetically related (Tojo et al., 1996; Panzacchi et al., 1997). Panzacchi et al (1997) also demonstrated an increased urinary albumin excretion after EPO injection in both Wistar-Kyoto rat (WKY) and spontaneous hypertensive rat (SHR) and this effect including hypertension in SHR was not modify by

ACEI. It seems that other factors rather than renin-angiotensin-aldosterone system might play a role in EPO induced hypertension and proteinuria in rats and human.

EPO can reduce glomerular fitration rate (GFR) and renal plasma flow (RPF) as seen in gentamicin induced renal failure in rats (Thongchai et al., 2008). Higher renal vascular resistanace and increased filtration fraction were found compared with rats treated with gentamicin alone. The study by Tojo et al. (1996) in SHR showed that EPO could reduce RPF without changing GFR. The reduction in RPF and hypertension was completely blocked by BQ-123, an endothelin ETA-receptor blocker. It is likely that the effect of EPO on vasoconstriction and renal functional changes in some species may be mediated by endothelin-1. In EPO overexpressing mice, the renal tissue especially tubular cell showed high concentration of endothelin-1 (ET-1) (Slowinski et al., 2002). These mice had high packed cell volume and high blood viscosity. In human, giving EPO to predialysis patients did not change blood pressure even anemia has been corrected (Tomczak-Watras et al., 2009). The renal hemodynamics including GFR index, blood flow rate index, renal vascular resistance index and filtration fraction remained the same. The results were similar to our study in which the dogs in group 2, which received EPO, and the dogs in group 3, which received EPO with ACEI, had no changes in blood pressure, urinary protein and electrolyte excretion and also renal function as expressed in term of plasma creatinine concentration.

AII is the agent responsible for increasing the glomerular sieving pore for protein leakage. Blocking AII with ACEI has been used to control protein loss in the urine. ACEI could lower proteinuria in human patients (Keilani et al., 1993; Maschio et al., 1994) and in rats with renal mass reduction (Buranakarl et al., 2003, Brooks et al., 1990.). We expected low proteinuria in the dogs in group 3. However, in group 3 the UPC was highest. The reason was due to veterinary practitioner who preferred to prescribe the ACEI in CKD cases in which animals had either hypertension or proteinuria.

The effect of EPO and EPO plus ACEI on blood pressure and renal function were also supported when measurement were performed in the same dogs before and after EPO administrations (Table 3). No significant changes in blood pressure, plasma creatinine concentration, urinary excretion of Na, K and protein were found. Dogs that received both EPO and ACEI showed no change in renal function and blood pressure compared with pretreatment value.

The packed cell volume in group 1 dogs with CKD before EPO administration was slightly lower than those recommended by Polzin et al. (1995)(Table 2). The low reticulocyte count although the animals were anemic suggested that the anemia was non-regenerative which was typical for chronic kidney disease. Anemia in chronic renal failure was primarily due to the failure of the kidney to produce EPO in response to hypoxia. A study in dogs with CKD showed that PCV declined as plasma creatinine rised (King et al., 1992; Buranakarl, et al., 2009). As PCV declined, plasma EPO rised. However, the EPO concentrations in uremic dogs were lower than expected for the degree of anemia (King et al., 1992). R-HuEPO can be used in dogs and cats with chronic kidney disease (Cowgill et al., 1998; Randolph et al., 2004). However, prolonged usage can produce the anti-EPO antibody resulting in resistant to human EPO and can develop aplastic anemia (Cowgill et al., 1998). The longest duration of EPO administration in this study was 40 days which may not be long enough to produce anti-EPO antibody. Thus, the PCVs, RBC count and reticulocyte count in group 2 and 3 were significantly higher than group 1. The degree of PCV and RBC count changes was slightly higher in group 2 compared with group 3. However, since some dogs may be injected with EPO in a different protocol and the protocol may be changed in each individual dogs, the average dose (intensity) was calculated and seemed to be higher in group 3 although PCV response was less. Thus, having ACEI along with EPO may modify PCV elevation. Therefore, the effect of EPO and ACEI was then performed in the same dogs before and after EPO administration with and without ACEI (experiment 2). The PCV increased significantly in the group receiving EPO alone ($p<0.01$) and in group receiving EPO and ACEI ($p<0.05$). However, the increase in PCV was more pronounced in the group receiving EPO alone ($p<0.05$) when compared with the same intensity (Fig 1). With the initial phase of EPO injection it seemed that the dogs receiving ACEI along with EPO required more EPO to achieve the same PCV as the group which did not received ACEI.

Many factors were evaluated and showed that the variability in EPO dosage at completion of stabilization phase could be account for by diabetes, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, proteinuria, transferin saturation, age, pre-treatment hemoglobin, geographical region, serum iron, and body mass index (Rossert et al., 2007). The correlation and odds ratio showed the association with EPO dosage at the start of the maintenance phase as well as with the EPO hyporesponsiveness. They showed that older age and greater BMI required more EPO. In this experiment, the age of the dogs varied, but most of them were old. The average age was similar in all 3 groups.

ACEI was one of the factor that required higher EPO. The data were supported by Qureshi et al (2007) who studied patients with chronic renal failure and received ACEI or angiotensin receptor antagonist and found that monthly increase in PCV was less in this group compared with patients received other kind of antihypertensive drug. Another study was performed in HD patients who received captopril and EPO for 10 month. He found that PCV in group receiving captopril was significantly lower even though patients received the same dose of EPO (Walter, 1993). However, controversy has been raised by many studies. One study was conducted in chronic HD patient who received ACEI for a long period of time (4 months). No significant changes were found in

average PCV of treated and untreated groups. Another study in HD patient who received 16 weeks on and 16 weeks off an ACEI showed that there were no differences in PCV (Conlon et al., 1994). A study in HD patient with negative finding of the effect of ACEI on PCV responding to EPO was also mentioned (Sanchez et al., 1995). It was interesting that the EPO and ACEI were given in human for a long period of time which was different from our study in which the dogs received ACEI for a short time during hospitalization.

In conclusion, giving EPO alone or in combination with ACEI at the initial phase for RBC production in CKD dogs did not modify the renal function, urinary loss of protein and systemic blood pressure. The PCV and RBC counts both increased significantly. However, at the first 15 days of treatment, dogs which received ACEI would required more EPO to increase PCV to the desired level compared with dogs receiving EPO alone.

Acknowledgement

This study was supported in part by the Special project for the 6th year student, Faculty of Veterinary Science, Chulalongkorn University.

References

Brooks, D.P., Contino, L.C., Trizna, W., Edwards, R.M., Ohlstein, E.H. and Solleveld, H.A. 1990. Effects of enalapril or the thromboxane receptor antagonist, daltroban, in rats with subtotal renal ablation. J Pharmacol Exp Ther. 253: 119-123.

Brown, S.A., Brown, C.A., Jacob, G., Stiles, J., Hendi, R.S. and Wilson, S. 2001. Effects of the angiotensin converting enzyme inhibitor benazepril in cats with induced renal insufficiency. Am J Vet Res. 62(3): 375-383.

Buranakarl, C., Ankanaporn, K., Thammacharoen, S., Trisiriroj, M., Maleeratmongkol, T., Thongchai, P. and Panasjaroen, S. 2007. Relationships between degree of azotemia and blood pressure, urinary protein: creatinine ratio and fractional excretion of electrolytes in dogs with renal azotemia. Vet Res Comm 31: 245-257.

Buranakarl, C., Kitjtawonrat, A., Pondeenana, S., Sunyasujaree, B., Kanjanapangka, S., Chaiyabutr, N. and Bovee, K.C. 2003. Comparison of dipyridamole and fosinopril on renal progression in nephrectomized rats. Nephrology 8: 80-91.

Buranakarl, C., Trisiriroj, M., Pondeenana, S., Tungjitpeanpong, T., Jarutakanon, P. and Penchome, R. 2009. Relationship between oxidative stress markers and red blood cell characteristics in renal azotemia dogs. Res Vet Sci. 86: 309-313.

Conlon P.J., Albers, F., Butterly, D. and Schwab, S.J. 1994. ACE inhibitors do not affect erythropoietin efficacy in haemodialysis patients. Nephrol Dial Transplant. 9: 1358. (Letters).

Cowgill, L.D., James, K.M., Levy, J.K., Browne, J.K., Miller, A., Lobingier, R.T. and Egrie, J.C. 1998. Use of recombinant human erythropoietin for management of anemia in dogs and cats with renal failure. J Am Vet Med Assoc. 212(4): 521-528.

Cruz, D.N., Perazella, M.A., Abu-Alfa, A.K. and Mahnensmith, R.L. 1996. Angiotensin-Converting enzyme inhibitor therapy in chronic hemodialysis patient: Any evidence of erythropoietin resistance? Am J Kidney Dis. 28 (4): 535-540.

Garcia, D.L., Anderson, S., Rennke, H.G. and Brenner, B.M. 1988. Anemia lessens and its prevention with recombinant human erythropoietin worsen glomerular injury and hypertention in rats with reduced renal mass. Proc Nat Acad Sci USA. 85: 6142-6146.

Jacob, F., Polzin, D.J., Osborne, C.A., Neaton, J.D., Kirk, C.A., Allen, T.A. and Swanson, L.L. 2005. Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. J Am Vet Med Assoc. 226(3): 393-400.

Jacob, F., Polzin, D.J., Osborne, C.A., Neaton, J.D., Lekcharoensuk, C., Allen, T.A., Kirk, C.A. and Swanson, L.L. 2003. Association between initial systolic blood pressure and risk of developing a uremic crisis or of dying in dogs with chronic renal failure. J Am Vet Med Assoc. 222(3): 322-329.

Keilani, T. Schlueter, W.A., Levin, M.L. and Batlle D.C. 1993. Improvement of lipid abnormalities associated with proteinuria using fosinopril, an angiotensin-converting enzyme inhibitor. Ann Intern Med. 118: 246-254.

King, L.G., Giger, U., Diserens, D. and Nagode, L.A. 1992. Anemia of chronic renal failure in dogs. Vet Intern Med. 6(5): 264-270.

Maschio, G., Cagnoli, L., Claroni, F., Fusaroli, M., Rugiu, C., Sanna, G., Sasdelli, M., Zuccala, A. and Zucchelli, P. 1994. ACE inhibition reduces proteinuria in normotensive patients with IGA nephropathy: A multicentre, randomized, placebo-controlled study. Nephrol Dial Transplant. 9: 265-269.

Miyashita, K., Tojo, A., Kimura, K., Goto, A., Omata, M., Nishiyama, K. and Fugita, T. 2004. Blood pressure response to erythropoietin injection in hemodialysis and predialysis patients. Hypertens Res. 27(2): 79-84.

Panzacchi, G., Pieruzzi, F., Castoldi, G., Busca, G., Bolla, G.B., Buccianti, G., Radice, F., Fava, C., Martini, I., Zanchetti, A., Golin, R. and Stella, A. 1997. Effect of erythropoietin administration on blood pressure and urinary albumin excretion in rats. Am J Hypertens. 10(7): 772-778.

Polzin, D.J., Osborne, C.A., Bartges, J.W., James, K.M. and Churchill, J.A. 1995. Chronic renal failure. In: Textbook of Veterinary Internal Medicine. S.J. Ettinger and E.C. Feldman (eds), Philadelphia: W.B. Saunders: 1734-1760.

Qureshi, I.Z., Abid, K., Ambreen, F. and Qureshi, A.L. 2007. Angiotensin converting enzyme inhibitors impair recombinant human erythropoietin induced erythropoiesis in patients with chronic

renal failure. Saudi Med J. 28(2): 193-196.

Randolph, J.E., Scaelett, J., Stokol, T. and MacLeod, J.N. 2004. Clinical efficacy and safety of recombinant canine erythropoietin in dogs with anemia of chronic renal failure and dogs with recombinant human erythropoietin-induced red cell aplasia. J Vet Intern Med. 18(1): 81-91.

Rossert, J., Gassmann-Mayer, C., Frei, D. and McClellan, W. 2007. Prevalence and predictors of epoetin hyporesponsiveness in chronic kidney disease patients. Nephrol Dial Transplant. 22: 794-800.

Sanchez, A.J. 1995. ACE inhibitors do not decrease rHuEPO response in patients with end-stage renal failure. Nephrol Dial Transplant. 10: 1476-1477 (Letters).

Slowinski, T., Schulz, N. and Ruschizka, F.T. 2002. Pattern of prepo-endothelin-1 expression revealed by receptor gene activity in kidney of erythropoietin-overexpressing mice. Clin Sci 103: 44S-47S.

Thongchai, P., Buranakarl, C. and Chaiyabutr, N. 2008. Renal function and oxidative stress following gentamicin induced renal injury in rats treated with erythropoietin, iron and vitamin E. Thai J Vet Med. 38(2): 19-27.

Tojo, A., Doumoto, M., Oka, K., Numabe, A., Kimura, K. and Yari, S. 1996. Endothelin-mediate effect of erythropoietin on blood pressure and renal hemodynamics in hypertensive rats. Am J Physiol (Reg Int Comp Physiol). 270(4): R744-R748.

Tomczak-Watras, W., Strozecki, P., Zuchora, Z., Szefer, J. and Manitius, J., 2009. Influence of the 6- month anemia therapy with erythropoietin on renal function and some hemodynamic parameters in predialysis patients. Pol Arch Med Wewn. 119(1-2): 45-51.

Walter, J. 1993. Does captopril decrease the effect of human recombinant erythropoietin in haemodialysis patients? Nephrol Dial Transplant 8: 1428 (Letters).