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Description of Clinicopathologic Changes during the Development and Clinical Resolution of Experimentally induced Canine Nephrotic Syndrome

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Abstract : The purpose of this study is to evaluate urine protein-to-creatinine ratio as a parameter for early detection of nephrotic syndrome and as a parameter for monitoring effectiveness in early course of treatment. Nine healthy dogs were sensitized by intravenous injection with 1 μg of endotoxin and 5 mg of native bovine serum albumin. After 1 week, 120 mg of cationized bovine serum albumin was injected intravenously 5 times a week. Among nine dogs, five dogs were confirmed as having developed glomerulonephritis and nephrotic syndrome by increase of urine protein-to-creatinine ratio(> 1.0), hypoalbuminemia (< 1.5 g/dl), hypercholesterolemia (> 240 mg/dl) and azotemia (BUN > 40 mg/dl). During the induction of glomerulonephritis and the progression to nephrotic syndrome, the increase of urine protein-to-creatinine ratio was firstly detected. 1 to 4 weeks later, hypoalbuminemia, hypercholesterolemia, and azotemia were detected. Prednisolone (2.2 mg/kg, bid) was administered orally to the dogs with induced nephrotic syndrome. In early stage of treatment, the increase of serum albumin and decrease of serum cholesterol were detected. 1 to 4 weeks later, decrease of urine protein-to-creatinine ratio was detected. It was concluded that urine protein-to-creatinine ratio is a useful parameter for early detection of nephrotic syndrome, and serum albumin and cholesterol are useful parameters for the monitoring in early course of treatment in nephrotic syndrome.

Key words : cationized bovine serum albumin, urine protein-to-creatinine ratio, albumin, cholesterol, prednisolone.

Introduction

The major glomerular diseases of dogs and cats are immune complex glomerulonephritis and amyloidosis. Both diseases can cause massive proteinuria and lead to progressive loss of functional renal mass[7]. Glomerulonephritis usually is caused by the presence of immune complexes in the glomerular capillary wall. These immune complexes initiate a series of events that can result in glomerular cell proliferation, thickening of capillary walls, glomerular hyalinization, and sclerosis. Irreversible damage to the glomerulus renders the entire nephron non-functional and, if the disease is progressive, results in renal failure. According to Macdougall (1986)[12], 52% of canine chronic renal diseases had glomerular and 48% non-glomerular diseases, and according to medical records of dogs with protein-losing glomerular disease, 77% had glomerulonephritis and 23% had amyloidosis[4].

Nephrotic syndrome is caused by glomerular disease and is characterized by proteinuria, hypoalbuminemia, hypercholesterolemia and edema[6,7]. The major cause of nephrotic syndrome in small animals is immune complex glomerulonephritis[6]. The normal glomerulus functions as both a size- and a charge-selective filter[9,10]. The filtration barrier is composed of three major components: the fenestrated endothelium of the glomerular capillary, the glomerular basement membrane, and the visceral epithelial cells (podocytes). The interdigitating foot processes of the podocytes and slit diaphragms between them are negatively charged because of the presence of acidic glycoproteins (glomerular polyanion)[7]. The basement membrane and endothelium also contain negatively charged glycoproteins. Thus circulating anionic proteins are repelled while cationic proteins are more likely to bind to the anionic sites and induce *in situ* immune complex glomerulonephritis[1]. Size-selective properties reside primarily in the basement membrane, which exclude by filtration macromolecules, such as albumin, greater than 34 nm in diameter[7].

The presence of glomerular polyanion restricts filtration of circulating negatively charged macromolecules and if the negative charge of glomerular membrane disappeares due to some mechanism, proteinuria develops. Because kidney is irreversible tissue, progression to glomerulonephritis and nephrotic syndrome is fatal.

There is no published study on experimentally induced canine nephrotic sydrome evaluated clinically, though there is a study on the immunofluorescence, histological and ultrastructural features of canine glomerulonephritis experimentally induced by charge-selective barrier theory[13]. We recognized that proteinuria, hypoalbuminemia, hypercholesterolemia, and edema are the characters of nephrotic syndrome. Thus, this study focused on evaluation of urine protein-to-creatinine ratio as a parameter for early detection of nephrotic syndrome and also as a parameter for monitoring effectiveness in early course of treatment.

Materials and Methods

Experimental animals

Breed, sex and body weight distributions were as follows.

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Breed: pug (n=2), shih tzu (n=3), Yorkshire terrier (n=1), maltese (n=1), mini-pin (n=1), mongrel (n=1)
Sex: male (n=7), female (n=2)
Body weight: 2.7 to 6.8
Age: 4 to 6 years

The dogs were each housed in individual cages and maintained on commercial dry dog food and water provided *ad libitum*.

Preparation of experimental animals

Nine dogs were determined to be healthy and have normal renal function as assessed by physical examination, CBC, serum biochemistry analysis, and urinalysis.

Before the experiment, prophylactic antibiotic (amoxacillin 22 mg/kg q12h) were administered to each dog for 3 days.

Nine healthy dogs were sensitized by intravenous injection with 1 μg of endotoxin (LPS) and 5 mg of native bovine serum albumin. After 1 week, 120 mg of cationized bovine serum albumin (C-BSA) was injected intravenously 5 times a week.

Prednisolone (2.2 mg/kg bid) was administered orally to the dogs with induced nephrotic syndrome. During the experiment, C-BSA was injected continuously, and serum biochemistry analysis and urine protein to creatinine ratios (UP/Cs) were measured weekly.

Urine protein determination

Pooled fresh canine serum was used as standard, after standardization by the biuret method. The protein standard was diluted with 154 mmol/L NaCl solution to give working standards of 250, 500, 1000 and 1500 mg/L. According to the methods of Lott et al. (1983)[11], 10 μl of specimen (standard or Urine) was added to 1 ml of coomassie brilliant blue (CBB) reagent. After 10 minutes, the absorbance at 465 and 595 nm was measured vs a water blank. The ratio of the absorbances, A595/A465, vs the concentration of the standards was plotted, on linear graph paper, and a standard curve was prepared each day. Specimens with protein concentrations exceeding 1500 mg/L were diluted with 154 mmol/L NaCl solution. If the protein concentration was less than 250 mg/L, the volume of the specimen was doubled.

Urine creatinine determination

Urine creatinine was determined by a creatinine test kit (MBL, Korea) using a modified Jaffe reaction by diluting the urine 1:100 in deionized water.

Blood collection and urine collection

Blood and urine samples were obtained between 10 AM and 2 PM. Blood samples were collected from either the cephalic or jugular vein. Urine samples were collected by voiding (midstream) or catheterization.

Urine and serum protein electrophoresis

Zone electrophoresis was carried out using a cellulose acetate membrane. A cellulose acetate membrane and buffer, sepraphore X (Gelman, USA) and high resolution buffer (Gelman) were used respectively. Electrophoresis was carried out according to the manufacturer's instructions.

Serum biochemistry analysis

Serum albumin, cholesterol, creatinine and BUN were determined by Selectra 2 (Merck, Netherland).

Monitoring the response of treatment

Prednisolone (2.2 mg/kg bid) was administered for treatment orally to the dogs with induced nephrotic syndrome. During treatment, serum biochemistry analysis and UP/Cs were measured weekly.

Results

Nine normal dogs were sensitized by intravenous injection with 1 μg of endotoxin (LPS) and 5 mg of native bovine serum albumin. After 1 week, 120 mg of cationized bovine serum albumin (C-BSA) was injected intravenously 5 times a week. Among nine dogs, five dogs were confirmed to have developed glomerulonephritis and nephrotic syndrome by increase of UP/Cs (>1.0), hypoalbuminemia (<1.5 g/dl), hypercholesterolemia (>240 mg/dl) and azotemia (BUN $>$ 40 mg/dl). Others (4 dogs) were died of anaphylactic shock before nephrotic syndrome induction, so they were excluded from this experiment.

Experimental animal 3 treated with C-BSA showed hypoalbuminemia, hypercholesterolemia, azotemia between 12 and 13 weeks. Before those, an increase of UP/C showed between 11 and 12 weeks. Prednisolone (2.2 mg/kg bid) was per orally administered twice a day from 13 week. An increase of serum albumin concentration and a decrease of serum cholesterol concentration showed immediately. After 2 weeks, a decrease of UP/C and a decrease of BUN showed (Fig 1).

Experimental animal 5 treated with C-BSA showed hypoalbuminemia, hypercholesterolemia between 11 and 12 weeks and azotemia between 13 and 14 weeks. Before those, an increase of UP/C showed between 10 and 11 weeks. Prednisolone (2.2 mg/kg bid) was per orally administered twice a day from 12 week. An increase of serum albumin concentration and a decrease of serum cholesterol concentration showed immediately. After 1 week, a decrease of UP/C and a decrease of BUN showed (Fig 2).

Experimental animal 6 treated with C-BSA showed hypoalbuminemia, hypercholesterolemia between 8 and 9 weeks and azotemia between 9 and 10 weeks. Before those, an increase of UP/C showed between 5 and 6 weeks. Prednisolone (2.2 mg/kg bid) was per orally administered twice a day from 9 week. An increase of serum albumin concentration and a decrease of serum cholesterol concentration showed immedi-

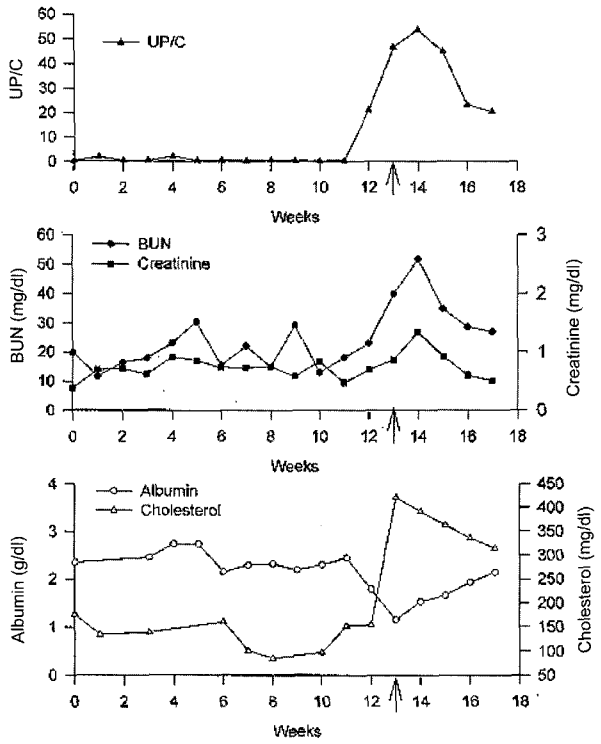

Fig 1. Change over time in UP/C, serum urea nitrogen, creatinine, albumin, and cholesterol of experimental animal 3 received cationized bovine serum albumin (120 mg/kg 5 times/ week, IV). Prednisolone 2.2 mg/kg was per orally administered twice a day from 13 week (↑).

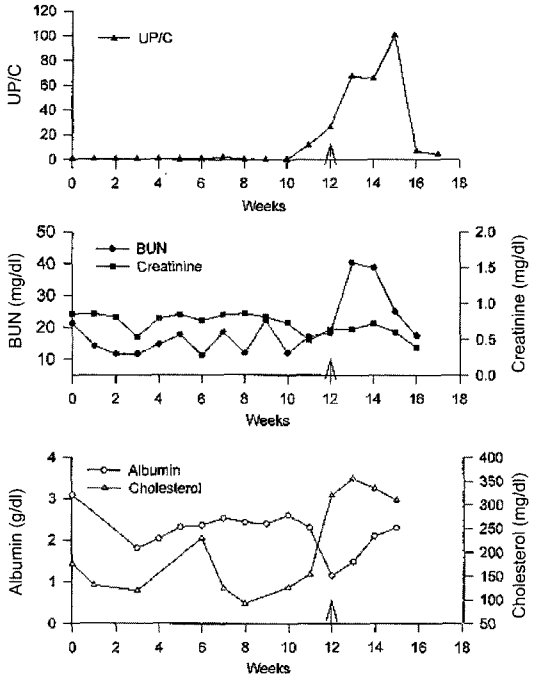

Fig 2. Change over time in UP/C, serum urea nitrogen, creatinine, albumin, and cholesterol of experimental animal 5 received cationized bovine serum albumin (120 mg/kg 5 times/ week, IV). Prednisolone 2.2 mg/kg was per orally administered twice a day from 12 week (↑).

ately. After 1 week, a decrease of UP/C showed and after 2 weeks, a decrease of BUN showed (Fig 3).

Experimental animal 8 treated with C-BSA showed hypoalbuminemia, hypercholesterolemia between 4 and 5weeks and azotemia between 5 and 6 weeks. Although mild hypercholesterolemia showed at 0 week, hypercholesterolemia showed clearly between 4 and 5 weeks. Before those, an increase of UP/C showed between 5 and 6 weeks. Prednisolone (2.2 mg/ kg bid) was per orally administered twice a day from 5 week. An increase of serum albumin concentration and a decrease of serum cholesterol concentration showed immediately. After 2 weeks, a decrease of UP/C and a decrease of BUN showed (Fig 4).

Experimental animal 9 treated with C-BSA showed hypoalbuminemia, hypercholesterolemia, uremia between 9 and 10 weeks and azotemia between 10 and 11 weeks. Before those, an increase of UP/C showed between 8 and 9 weeks. Prednisolone (2.2 mg/kg bid) was per orally administered twice a day from 10 week. An increase of serum albumin concentration and a decrease of serum cholesterol concentration showed immediately. After 1 week, a decrease of UP/C and a decrease of BUN showed (Fig 5).

During both the induction of glomerulonephritis and the progression to nephrotic syndrome, the increase of UP/C was

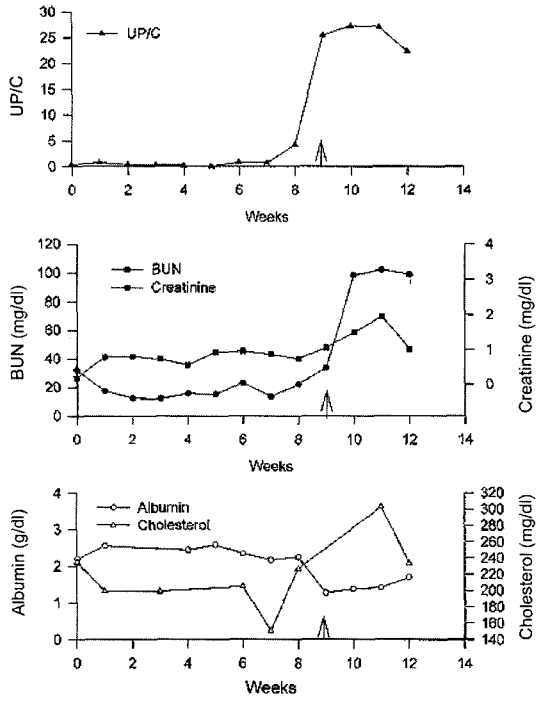

Fig 3. Change over time in UP/C, serum urea nitrogen, creatinine, albumin, and cholesterol of experimental animal 6 received cationized bovine serum albumin (120 mg/kg 5 times/week, IV). Prednisolone 2.2 mg/kg was per orally administered twice a day from 9 week (↑).

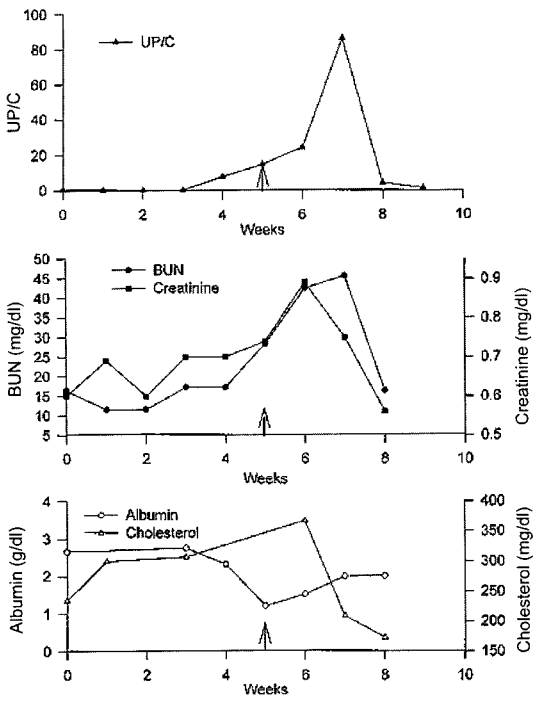

Fig 4. Change over time in UP/C, serum urea nitrogen, creatinine, albumin, and cholesterol of experimental animal 8 received cationized bovine serum albumin (120 mg/kg 5 times/week, IV). Prednisolone 2.2 mg/kg was per orally administered twice a day from 5 week (↑).

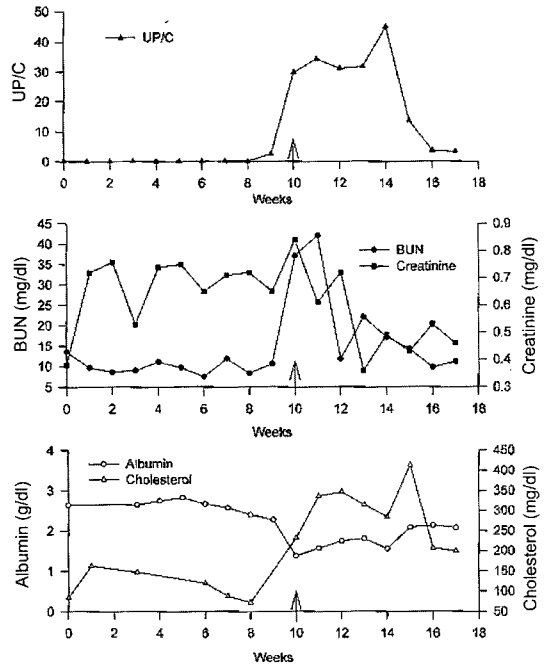

Fig 5. Change over time in UP/C, serum urea nitrogen, creatinine, albumin, and cholesterol of experimental animal 9 received cationized bovine serum albumin (120 mg/kg 5 times/week, IV). Prednisolone 2.2 mg/kg was per orally administered twice a day from 10 week (↑).

firstly detected. 1 to 4 weeks later, hypoalbuminemia, hypercholesterolemia, and azotemia were detected. Prednisolone (2.2 mg/kg bid) was administered orally to the dogs with induced nephrotic syndrome. In early stage of treatment, the increase of serum albumin and decrease of serum cholesterol were detected. 1 to 4 weeks later, decrease of UP/C was detected.

Discussion

In the immunologic mechanisms of glomerular injury, circulating soluble immune complexes become trapped in the glomerular filter and have fixed complement. Chemotactic complement components attract neutrophils to the area, and the release of free oxygen radicals and lysosomal enzymes from the neutrophils has resulted in damage to the glomerulus[7]. Non-glomerular antigens may localize in the glomerular capillary wall as a result of electric charge interaction or biochemical affinity with the glomerular basement membrane[7]. The basement membrane and endothelium contain negatively charged glycoproteins. Thus circulating anionic proteins are repelled while cationic proteins are more likely to bind to the anionic sites and induce *in situ* immune complex glomerulonephritis[1]. This is the theoretical basis of this experiment.

According to the standard of Center (1985)[2], a dog which shows an increase of UP/C (> 1.0), hypoalbuminemia (< 1.5 g /dl) is diagnosed as having nephrotic syndrome. Examination of a 24 hr collection of urine is the method of choice for quantitative proteinuria. Errors caused by variation in protein concentration due to changes in urine specific gravity are minimized by 24 hr urine collection. Such collections, however, require the use of a metabolic cage or an indwelling urinary catheter, making the procedure cumbersome and expensive to do. In addition, incomplete collection of all urine produced over the 24 hr period will result in errors[8]. Recently, it was shown that in dogs, a strong correlation existed between the total 24 hr urine protein excretion and the UP/C from a single urine sample. Creatinine is produced at a constant rate, freely filtered by the glomeruli, and is not significantly secreted or reabsorbed by the renal tubules. Consequently, the concentration of creatinine in urine is proportional to the total solute concentration of the urine, and dividing the urine protein concentration (mg/dl) by the urine creatinine concentration (mg/dl) eliminates the effect of urine volume on urine protein concentration[5,7].

Hypercholesterolemia and hyerlipidemia associated with nephrotic syndrome probably are caused by a combination of increased hepatic synthesis and decreased catabolism of proteins and lipoproteins. Large-molecular-weight, cholesterol-rich lipoproteins that are not easily lost through the damaged glomerular capillary wall accumulate, whereas smaller-molecular-weight proteins, such as albumin and anti-thrombin III, are lost in the urine. In nephrotic patients, plasma albumin concentrations are inversely correlated with plasma cholesterol concentration, and cholesterol and lipid concentrations

increase as albumin concentration decreases. Decreased plasma albumin concentration is thought to stimulate hepatic synthesis of very low density lipoproteins. At the same time, there is decreased hepatic catabolism of lipoproteins associated with abnormal lipoprotein lipase function. Hypercholesterolemia (> 240 mg/dl) has been observed in 60 per cent of dogs with glomerulonephritis[3]. If the disease is progressive, it results in decrease of GFR, azotemia and renal failure[7].

Prednisolone (2.2 mg/kg bid) was administered orally to the dogs with induced nephrotic syndrome. In early stage of treatment, the increase of serum albumin and decrease of serum cholesterol were detected. 1 to 4 weeks later, decrease of urine protein-to-creatinine ratio was detected. The alleviation of proteinuria by administration of prednisolone alone in experimental animals with nephritis suggested that glomerulonephritis was immune-mediated. Our study revealed that the data of serum albumin and cholesterol are more useful parameters than those of UP/C for monitoring early course of treatment in nephrotic syndrome. This study is not statistical but descriptive, because the number of experimental animals is small and the induction period and progression of the experimental animals are different.

Further study is necessary to determine whether or not same results are obtained in dogs naturally occurred nephrotic syndrome. It was concluded that urine protein-to-creatinine ratio is a useful parameter for early detection of nephrotic syndrome, and serum albumin and cholesterol are useful parameters for the monitoring in early course of treatment in nephrotic syndrome.

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실험적으로 유발한 개 신증후군의 진행 및 치유과정에서의 임상병리학적 변화

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이 연구의 목적은 실험적으로 유발한 면역매개성 사구체 질환이 신증후군으로 진행하는 과정과 치유과정에서 단백뇨 배설에 근거한 조기진단 및 치유과정의 감시에 대한 평가를 하는 것이다. 두당 endotoxin 1 μg과 native bovine serum albumin 5 mg을 9두의 건강한 개에 정맥주사하여 감작시키고, 그 후 1주일부터 양이온화한 bovine serum albumin 120 mg을 주 5회 반복 정맥주사한 결과 5두에서 면역매개성 사구체신염 과 신증후군이 발생하였다. 사구체신염이 발생한 실험동물에서는 요단백질/ 크레아티닌 비의 증가 (>1.0), 저알부민혈증 (<1.5 g/dl), BUN 증가 (>40 mg/dl), 고콜레스테롤혈증 (>240 mg/dl), 부종 등이 나타났다. 이것은 임상병리학적으로 평가 가능한 면역매개성 사구체신염 및 신증후군 모델견이 제작되었음을 제시하는 것이었다. 사구체신염의 유발과 신증후군의 진행과정에 요단백질/크레아티닌 비의 증가가 저알부민혈증, BUN 증가, 고콜레스테롤혈증보다 1-4주 먼저 출현하였다. 사구체신염에 의해 신증후군이 유발된 실험동물 모두에서 prednisolone을 2.2 mg/kg씩 1일 2회 경구투여한 결과 혈중 알부민 농도의 증가와 콜레스테롤 농도의 감소가 먼저 일어나고, 그 후 1-4주에 요단백질/크레아티닌 비가 감소하기 시작하였다. 종합적으로 볼 때, 면역매개성 사구체신염성 신증후군의 조기진단에는 요 단백질/크레아티닌 비가 유리한 지표로, 그리고 초기 치유과정의 감시에는 혈청 알부민, 콜레스테롤이 더 유리한 임상 지표로 이용될 수 있음을 확인하였다.

주요어 : 사구체신염, 양이온화 bovine serum albumin, 요단백질/크레아티닌 비, 신증후군