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Surgical induction of experimental chronic renal failure in the sheep

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ABSTRACT

The kidneys excrete the end-products of tissue metabolism and maintain fluid, electrolyte and acid-base balance. Renal failure is the result of impaired renal excretory and homeostatic functions and the clinical syndrome of uremia will be present. The pathophysiology of uremia, however, is difficult to study in humans because of the type of experimentation required. The aim of this study was to induce experimental model of chronic renal failure in sheep for further research. Uremic state was created by two stages surgery in eight adult male sheep. Initially, in the left kidney, the posterior branch of the renal artery was completely ligated. At least 3 weeks after the animal had recuperated from the renal infarction, the right kidney was removed. All sheep became uremic and mildly anemic. Four weeks after right nephrectomy (second stage of surgery) mean urea, creatinine, potassium, and sodium levels in the serum of sheep were significantly elevated and were 110.4 ± 11.69 mg/dl, 3.09 ± 0.84 mg/dl, 4.8 6 ± 0.40 mmol/L and 130.5 ± 8.02mmol/L, respectively. Mean PCV of sheep at same time was 25.2 percent. Acute renal failure developed in one sheep so that serum biochemical parameters including urea, creatinine, potassium, and sodium levels increased to 250 mg/dl, 7.3 mg/dl, 7.4 mmol/L and 123 mmol/L, respectively. This study suggested that two stages subtotal nephrectomy can be used for experimentally induction of chronic renal failure and uremia in sheep.

Key words: *Experimental Chronic Renal Failure, Nephrectomy, Sheep, Uremia*

INTRODUCTION

The pathophysiology of the abnormalities associated with renal dysfunction, however, is difficult to study in human because of the type of experimentation required. Therefore animal models which have been the mainstay of experimental means to study chronic renal failure have been developed to investigate these problems. An ideal experimental animal model provides a stable uremic milieu to allow experimental manipulation. A wide range of experimental (dietary and pharmacologic) therapies explored in such models have also proven to be clinically efficacious. Three main ways of inducing experimental uremia include surgical five-sixths nephrectomy, vascular ligation method and chemical nephrectomy[3]. Numerous methods, albeit not well standardized, are available for inducing the model of chronic renal failure and each has been strictly defined in a given animal species [4,6,7,8,13]. None of them can ever duplicate the original condition in a human kidney. Because of the need to study certain

aspects of CRF in an experimental setting, we have established a large animal model for this syndrome in the sheep. The purpose of this study was to monitor the nephrotoxic effects of ischemia in sheep.

MATERIALS AND METHODS

Experimental animals:
Eight clinically healthy, yearling male Ghezel (Iranian fat tailed) sheep and weighing 40-45 kg were used. All animals were kept indoor in group box during the whole experimental period, under similar conditions and manual feed including alfalfa, barley, treated wheat straw (treated with 5% urea, 2.5% molasses and 2% salt)and drinking water was given ad libitum for several weeks before the trial. After deworming of sheep with Albendazol 5% (Dieverm®, 15 mg/kg, PO, Damloran Razak pharma, Lorestan, Iran) and Ivermectin 1% (Intermectin®, Interchemie, Holland, 200µg/kg, SC) animal were prepared for experiment. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care, and Ethics Committee of Faculty of Veterinary Medicine, Islamic Azad University approved the protocol.

Anesthetic and postoperative management:
The animals were fasted 12 h before surgery. General anesthesia was induced with combination of Xylazine hydrochloride (Xylazine 2%, Alfasan, Worden-Holland, 11 mg/kg, IM) and Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 0.22 mg/kg, IM). Local anesthesia was performed using Lidocain 2% (Alfacaïne + adrenaline®, Alfasan, Woerden-Holland). Postoperative management consisted of administration of antibiotic and non-steroidal anti-inflammatory drug. After each stage of surgery, two injection of penicillin mix 2:1:1 (benzathine:procaine:potassium) with 2 days interval and ketoprofen (3 mg/kg, daily, for 3 days) were injected intramuscularly.

Surgical procedures:
After induction of general anesthesia, animals were restrained in lateral recumbency and right flank was routinely prepared for surgery. Then uremic state was created by two stages surgery. In sheep, the renal artery divides at the renal hilus into posterior and anterior branches (Figure 1) that supply approximately two thirds and one third of the kidney, respectively. Initially, in the left kidney, the posterior branch of the renal artery was completely ligated. For this aim, an incision at the right mid-paralumbar fossa to ligation of the left kidney branch was used. At least 3 weeks after the animal had recuperated from the renal infarction, the right kidney was removed. Another incision is made just caudal and parallel to the last right rib to remove the right kidney. This intervening time allowed the renal remnant to hypertrophy and reduced the likelihood of sudden death due to acute renal failure immediately post nephrectomy. This method has been described in mice, previously [5].

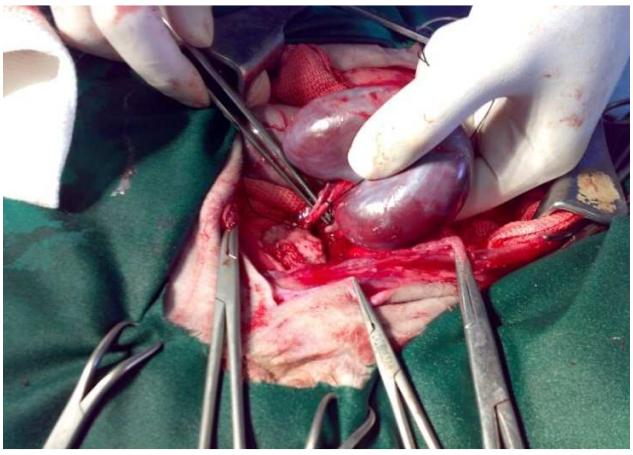

Figure 1: Left kidney arteries ligation (first stage of surgery)

In one of cases (case no. 3), due to excessive and uncontrollable bleeding from left kidney vessels, total nephrectomy was done in that kidney. Three weeks later the remaining right kidney was removed completely after ligation of the renal artery, vein, and ureter.

Sampling and laboratory procedures:
Regularly blood samples were collected weekly before and after surgery, throughout the study. For hematological and biochemical analysis, approximately 5 mL blood samples were collected from jugular vein into EDTA and serum tubes. Packed cell volume (PCV) measured by routine procedures. Sera were analyzed for urea, creatinine, albumin by an automatic analyzer (Alcyon^{TM} 300, Abbott lab., Illinois, USA), using commercial kits (Pars Azmoon Co. INC., Karadj, Iran). Also serum potassium (K) and sodium (Na) value were quantified by Flame photometric method.

Statistical analysis:
All statistical analyzed by Statistical Package for Social Sciences for Windows, version 17.0 (SPSS Inc.).Data normality was tested by Kolmogorov-Smirnov test. Analysis of variance (ANOVA) test was used for comparison of measured factors in various times. All values were expressed as mean and standard deviation (SD), and $P<0.05$ was considered as statistically significant.

RESULTS

There was no marked deterioration in the clinical condition of the animals after the first stage of surgery. Mild and transient pain was the most common finding was seen after ligation of left renal artery branches, which was manifested as intermittent tooth grinding, continued for 3 days. But after the second surgery, the right nephrectomy, general condition of animals was not normal; they were depressed, anorectic and water intake was considerably reduced. They were also showed pain signs, obviously. One sheep (case no. 2) died suddenly at third day, others survived 15 to 120 days.

In the second stage of surgery, the left kidneys that had been ligated were studied. Fibrotic and small irregular kidneys were evident (Figure 2). Comparison of left kidney size at day 0, and 3 days after right nephrectomy, measured by ultrasonography, confirmed a considerable reduction in its width and length. Renal fibrosis was most evident in dorsal part of ligated left kidney, and pathological change was few in ventral part.

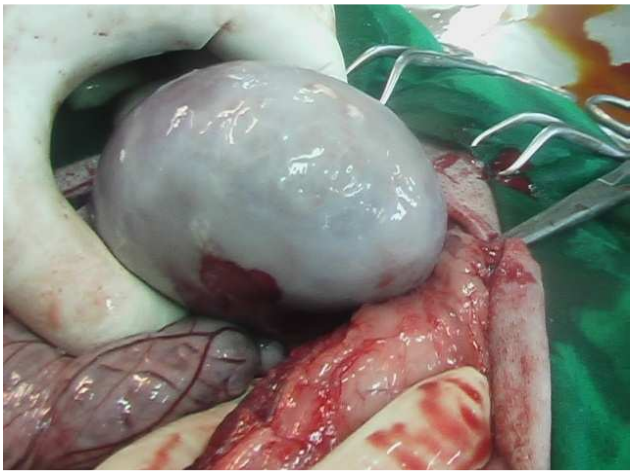

Figure 2: Renal fibrosis in left kidney two weeks after second stage of surgery

The changes in blood constituents are shown in Table 1.All sheep became uremic and mildly anemic. Blood urea and serum creatinine concentrations showed a progressive increase which was significant at all stages of observation. The serum levels of urea (P=0.004), creatinine (P=0.002), and potassium (P=0.037) and sodium (P=0.048), significantly increased four weeks after right nephrectomy (Figure 3). Mean packed cell volume of sheep at same time significantly lower previous times of experiment (P=0.031) (Figure 4). Serum biochemical parameters were not significantly different between case no. 3 and others. One of eight sheep (case no. 4) died two weeks after right nephrectomy. One day before his death, serum biochemical parameters including urea, creatinine, potassium, and sodium levels had been increased to 250 mg/dl, 7.3 mg/dl,7.4mmol/Land 123mmol/L, respectively. At necropsy, left kidney was scarified and smaller than normal size. The size of left kidney before surgery was 76 mm in length and 41 mm in width that decreased to 53 mm and 35 mm, respectively.

Table 1.PCV and blood chemistries in normal and uremic sheep (mean ± SD)

Time (week)	Urea (mg/dL)	Creatinine (mg/dL)	Potassium (mmol/dL)	Sodium (mmol/dL)	Alb (g/dL)	PCV (%)
0 (after deworming)	28.75 ± 6.07	0.88 ± 0.12	4.07 ± 0.53	119.50 ± 2.51	3.41 ± 0.22	34.5 ± 2.0
1(1st surgery)	25.6 ± 6.84	0.79 ± 0.23	3.80 ± 0.84	125.40 ± 6.10	3.67 ± 0.32	35.0 ± 1.8
2	101.8 ± 41.37	3.01 ± 2.47	4.78 ± 1.64	134.40 ± 3.36	3.72 ± 0.27	34.6 ± 1.7
3	63.2 ± 7.22	1.81 ± 0.16	5.02 ± 0.49	128.50 ± 8.69	3.74 ± 0.63	33.8 ± 3.2
4 (2nd surgery)	53.5 ± 5.67	1.63 ± 0.18	5.13 ± 0.20	128.67 ± 3.73	4.00 ± 0.35	33.9 ± 3.0
5	102.4 ± 14.77	2.88 ± 0.53	5.06 ± 0.66	129.00 ± 5.56	3.90 ± 0.23	30.4 ± 2.2
6	87.0 ± 25.27	1.80 ± 0.09	4.96 ± 0.43	143.67 ± 3.05	4.02 ± 0.27	29.9 ± 1.6
7	95.6 ± 10.03	2.76 ± 1.93	4.80 ± 0.55	138.33 ± 12.89	3.85 ± 0.35	28.4 ± 4.1
8	110.4 ± 11.69	3.09 ± 0.84	4.86 ± 0.40	130.50 ± 8.02	3.74 ± 0.28	25.2 ± 3.1
9	132.0 ± 10.81	3.60 ± 0.44	5.29 ± 0.94	132.00 ± 7.07	3.92 ± 0.14	25.8 ± 2.7
10	153.8 ± 8.46	3.90 ± 0.68	6.10 ± 1.22	126.33 ± 2.82	3.96 ± 0.07	26.5 ± 3.0
11	147.0 ± 8.92	3.49 ± 0.51	5.86 ± 0.40	131.50 ± 6.65	3.83 ± 0.12	27.2 ± 3.8
12	144.8 ± 7.26	3.42 ± 0.30	4.86 ± 0.75	131.25 ± 9.19	3.77 ± 0.26	27.0 ± 3.1
13	146.3 ± 4.11	3.31 ± 0.46	4.43 ± 0.63	130.39 ± 6.23	3.89 ± 0.32	28.3 ± 2.5

No significant change was seen in the levels of serum albumin (P=0.057). There were significant correlation between serum urea and creatinine (P=0.000, r=0.998), urea and potassium (P=0.001, r=0.501), urea and sodium (P=0.016, r=0.364), creatinine and potassium (P=0.002, r=0.447), and between creatinine and sodium (P=0.019, r=0.362).

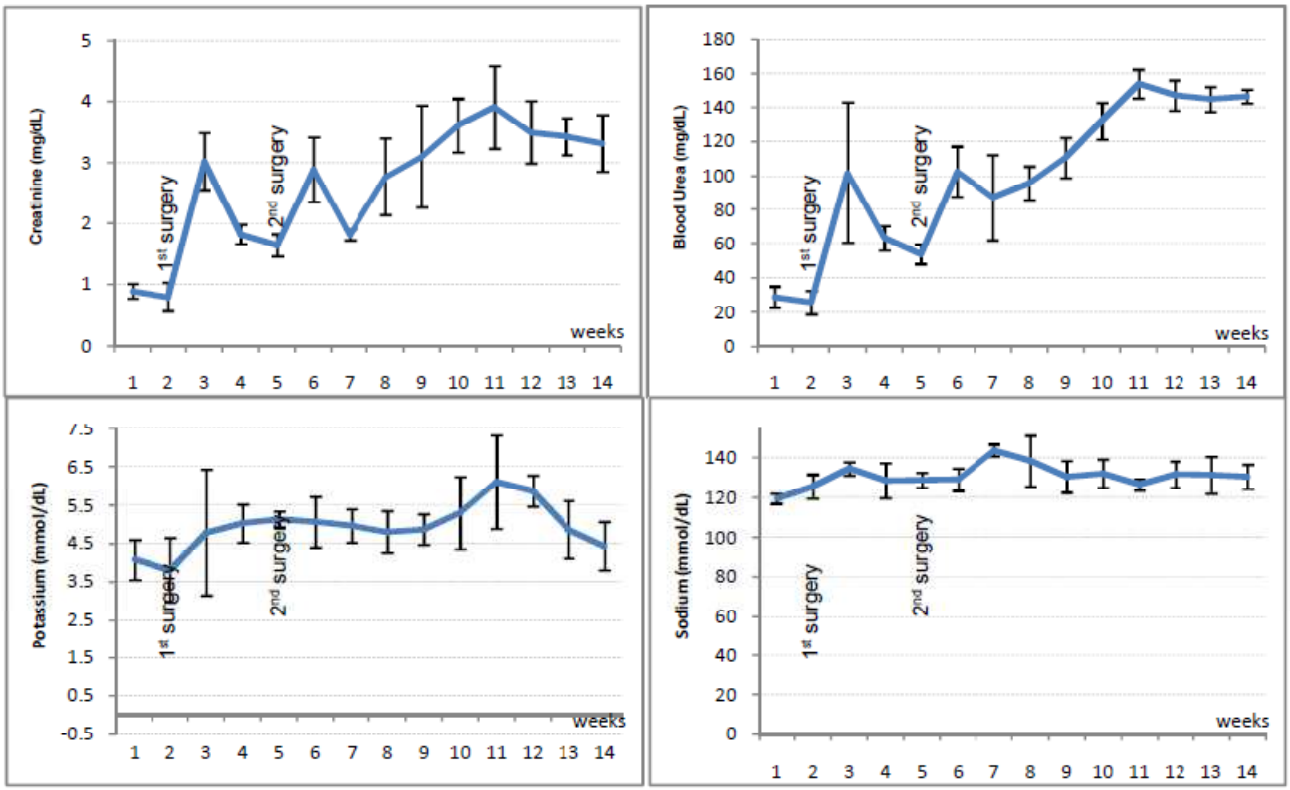

Figure 3: Serum levels of urea, creatinine, potassium and sodium in sheep with experimental CRF

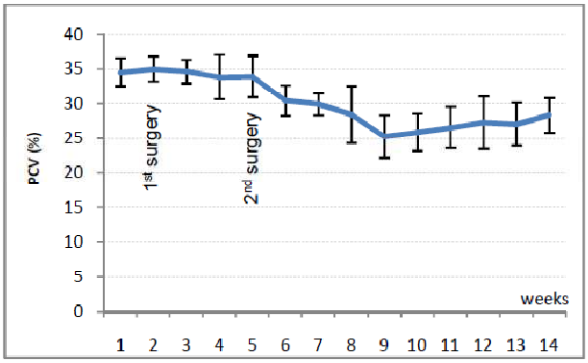

Figure 4: Packed cell volume in sheep with experimental CRF

DISCUSSION

A large-animal model of chronic renal failure (CRF), produced by subtotal nephrectomy, has been created specifically to study the pathophysiologic changes of CRF. Serum concentrations of urea and creatinine do not rise appreciably above the normal range until 60-75% of nephrons are destroyed [10].Progressive increase of serum urea concentration was accompanied by creatinine elevation in all animals. These changes are predominant abnormalities in most species with renal failure [12,14,15,16].

The plasma potassium values increased after nephrectomy in these sheep. Mildly increases in serum potassium concentrations after bilateral nephrectomy had been reported in sheep [11,12] and cattle [15,16]. Hyperkalemia is on one the important index of renal failure and dialysis but failure of the progressive rise in the plasma potassium values in ruminant after total nephrectomy was assigned to more excretion of this ion via the saliva [15,16]. Since we did not analyze the saliva potassium content, a correlation is difficult to establish. Additionally plasma potassium concentrations quite variability had been reported in azotemic cattle with various disease conditions [1].Serum albumin was mildly increased in uremic sheep but wasn't significant. This finding, which was attributed to decrease water consumption and dehydration, is contrary to long-held beliefs that renal failure cause hypoproteinemia secondary due to proteinuria and glomerulosclerosis [3].Decrease in packed cell volume might be due to renal dysfunction and emphasize the kidney roles in production of erythropoietin.

Urinalysis is an essential component of the examination of the urinary system and a method had been described for steer, recently [2]. But total urine collection from sheep wasn't successful in this experiment. Objective observation of sheep pens revealed that oliguria occurred shortly after surgeriesand polyuria occurred 4 weeks after second stage of surgery that cause bed wetness.

Preferably, the sheep should be maintained in stable chronic renal failure for relatively long periods of time for experiments of long-term design. To illustrate these criteria in animal model selection, a total nephrectomy model (an anephric model) would not qualify as an ideal long-term model because the most animals would survive for less than 100 hours without dialysis support [3,12]. Experimental uremia has also been induced by surgical removal of both kidneys but the results, especially in ruminants, are quite different from those in naturally occurring renal failure. The clinical pathology is similar but there is a prolonged period of normality after the surgery [10]. Selective ligation of the renal arteries provides an alternative method of inducing experimental uremia. Briefly, unilateral (right) nephrectomy is combined with ligation of most of the primary and secondary divisions of the main left renal artery. The degree of renal infarction is assessed by discoloration (for example, 75% of the kidney) and, hence, is interpreted as ischemia that progresses to infarction. The feasibility of achieving a reproducible chronic renal failure model by vascular ligation, even in the case of larger animals, is also compromised by the formation of collateral vessels that bypass the ligated branches and the inconsistent ramification pattern of the renal artery [14].

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