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Original Paper

Effect of Renal Artery Ligation on Serum Lipids in Rats

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Key words: renal artery ligation, serum lipid, serum cholesterol, hyperlipidemia

Abstract

The effects of a renal artery ligation (RAL) on longevity and age related changes in blood biochemical parameters were studied in the Sprague-Dawley rat.

The blood urea nitrogen and creatinine levels were already high in the RAL group four weeks after surgery, and were more than double the values of the control rats by the 48th week. Total cholesterol, esterified cholesterol and free cholesterol levels were also higher in the RAL rats after the 4th week. Compared with controls, the average percent of high density lipoprotein cholesterol gradually decreased and the percent of low density lipoprotein cholesterol increased following RAL surgery.

These findings suggest that an unilateral ligation of the renal artery causes abnormal metabolism of proteins and lipids, promotes uremia and hyperlipidemia due to under compensation by the remaining kidney, and may be a factor in shortening the life span.

Introduction

Various animals, including humans, can survive and lead a normal life with only one kidney due to a congenital absence of the contralateral organ[1]. The Japanese Society for Dialysis Therapy reported that 219,183 people underwent dialysis therapy during 2001, most commonly due to diabetic nephropathy (38.1%) and chronic glomerular nephritis (32.4%). It has also been reported that the frequency of renal failure caused by nephrosclerosis has been increasing, along with varying degrees of stenosis and obstruction in the renal artery[2]. Life can be maintained with a single kidney, not only after an accident or transplant, but also as a result of nephrosclerosis. It has already been reported that renal artery ligation (RAL) stimulates plasma renin and causes high blood pressure[2-6]. However, unilateral nephrectomy results in a rapid compensatory response of the contralateral kidney[7]. It was also reported that patients with little kidney tissue remaining show incipient progressive renal disorder and are at high risk of glomerulonephritis[8]. However, there are few reports on the outcomes after RAL, which is eventually fatal.

The authors have reported on the life span and changes in the blood biochemistry of rats with RAL[9]. The results showed that uremia often causes hyperlipidemia[10,11]. Therefore, in the present study, serum lipids in rats with RAL were compared with those of normal rats.

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Materials and Methods

1. Experimental animals and maintenance conditions

Thirty-four seven week old male Sprague-Dawley rats (200∼250g) were fed a solid diet (MF Oriental Yeast Co., Ltd., Tokyo) for one week prior to the start of the experiment. They were divided at random into two groups : nine control rats (BW 299.4±13.6g) that underwent a sham-operation and 25 rats (BW 275.3±8.7g) that underwent ligation of the left renal artery and vein in a single procedure under Nembutal (50 mg/kg BW) anesthesia.

Feeding of the two groups was started at almost the same time after surgery. The rats were given food and water *ad libitum* and kept in cages (34 × 36 × 51.5cm) in groups of three. They were housed at a room temperature of 22±3 ℃ at a relative humidity of 55±3%, and under a 12/12-h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.).

2. Measurement of body weight

The BW was measured once a week until death. After death, the abdomen was incised to confirm that the ligation of the left renal artery was intact.

3. Serum biochemistry analyses

Blood samples were collected from the tail vein under ether anesthesia after a 17 hour fasting period. Samples were collected immediately before the operation and on the 4th, 12th, 24th, 48th and 72nd weeks after surgery. The serum was centrifuged for 15 min at 3,000 rpm and kept at -40 ℃ until analysis.

Levels of the following biochemical parameters were measured with a Hitachi 101 model blood chemistry analyzer: blood urea nitrogen (BUN, using the Urease UV method), creatinine (Crn, using the Folin-Wu method), total cholesterol (T-Cho, using the Enzyme method), esterified cholesterol (E-Cho, using the Enzyme method), free cholesterol (F-Cho using the Enzyme method), free fatty acids (FFA, using the Enzyme method), high density lipoprotein choresterol (HDL-Cho, using the Enzyme method), low density lipoprotein cholesterol (LDL-Cho, using the Enzyme method), triglyseride (TG, using the Enzyme method), and phospholipid (PL, using the Enzyme method).

4. Statistical assessment of the results

Values are expressed as mean±SD.

Statistical analysis was carried out by the Smirnov method.

Results

1. Growth curve

The growth curves of the RAL and control groups are shown in Fig. 1. The average body weight in the RAL group increased more rapidly until the 35th week.

2. Food intake

The composition of the diet and daily food intakes are shown in Table 1 and Table 2.

The food intakes in the RAL group were significantly higher ($p<0.05$) than those in the control group at the 4th and 12th weeks after surgery.

3. BUN and Crn

The changes in BUN and Crn levels are shown in Fig. 2. BUN levels in the RAL group were significantly higher ($p<0.01$) than those in the control group after the 4th week, and by the 48th week, they were more than double those in the control group. Crn, an indicator of renal function, also was significantly elevated ($p<0.05$) at the 4th week in the RAL group, and the difference was more pronouncesd ($p<0.01$), after the

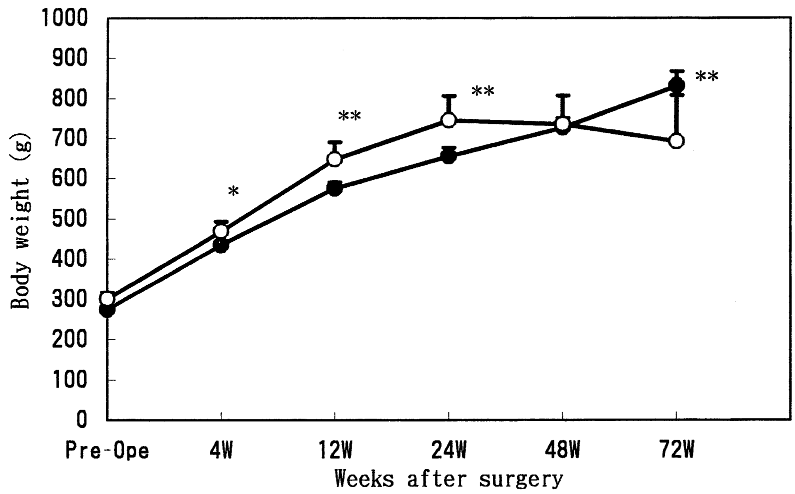

Fig. 1 Growth curves of control rats and rats with RAL. ●, control rats (start n=9); ○, rats with RAL (start n=25). Values are the mean ± SD. Significantly different from control rats: *$p<0.05$, **$p<0.01$.

Table 1 Composition of the diets

(g/100g)	MF
Protein	23.6
Fat	5.3
Ash	6.1
Cellulose	2.9
Nitrogen-free extract	54.4
Water	7.7
Total	100.0
(Energy	360kcal/100g)

Table 2 Daily food intake of control rats and rats with RAL

(g/day)	Control	RAL
Pre-Ope	25.7±3.8	26.8±5.3
4W	25.3±4.4	33.5±5.4*
12W	23.4±1.3	32.6±6.1*
24W	28.7±2.6	33.6±7.4
48W	30.8±6.5	33.2±6.9

Values are the mean ±SD.
Significantly different from control rats : *$p<0.05$.

48th week. In the control group, no change in BUN or Crn was noted until the 72nd week.

4. T-Cho, E-Cho, F-Cho, HDL-Cho and LDL-Cho

T-Cho, E-Cho, F-Cho, HDL-Cho and LDL-Cho results are shown in Fig. 3. T-Cho, E-Cho and F-Cho levels in the RAL group were significantly higher than in the control group after four weeks.

At the 4th week, the RAL group had a significantly ($p<0.05$) lower HDL-Cho percent, and a significantly higher LDL-Cho percent ($p<0.05$) than the control group, by the 48th week.

5. TG, PL and FFA

TG, PL and FFA results are shown in Fig. 4. TG and PL levels in the RAL group were lower after 24 weeks. However, by the 48th week, they appeared to be higher than the control group.

FFA levels gradually decreased after the operation in both groups. By the 12th week, the RAL group had significantly higher mean FFA levels, but they were significantly lower than the control group by the 48th week.

Discussion

Life can be maintained with a single kidney, not only after accidents or transplants, but also as a result of nephrosclerosis. RAL rats were considered to be analogous to the uninephrectomy model. The long-term functional changes in the intact kidney after renal artery ligation are still not completely known.

However, it was previously reported[9] and shown again in Fig. 1 that the survival rate of rats with a renal artery ligation was shorter than control rats. A 50% survival rate was observed at the 92nd week in

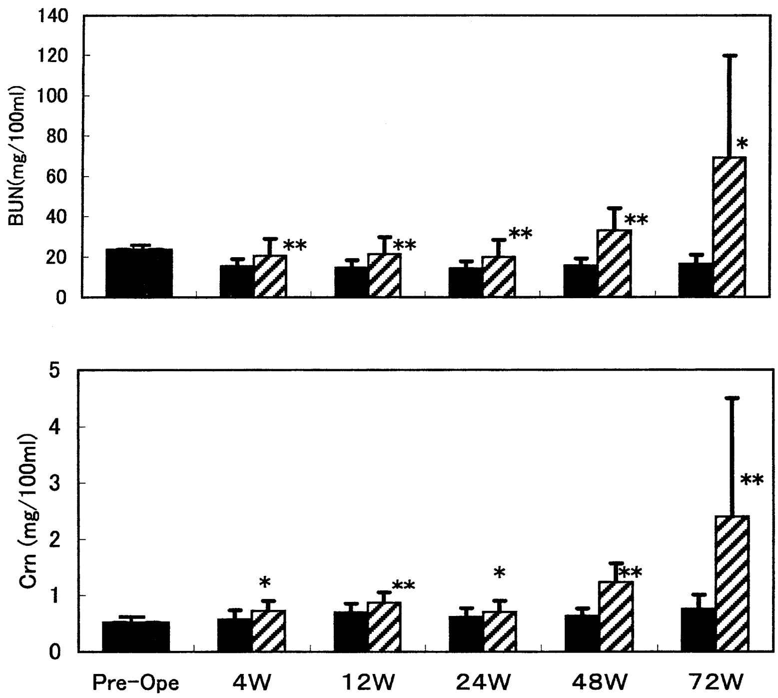

Fig. 2 Changes of serum BUN and Crn levels in control rats and rats with RAL. ■, Control rats; ▨, rats with RAL. Values are the mean ± SD. Significantly different from control rats: *$p<0.05$, **$p<0.01$.

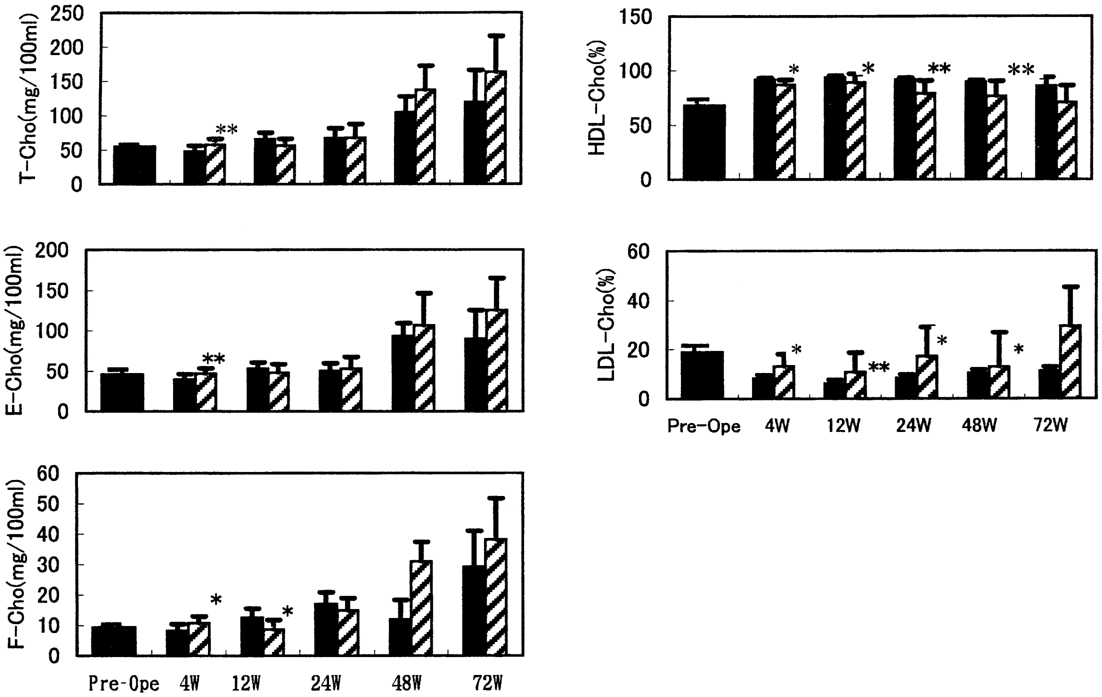

Fig. 3 Changes of serum T-Cho, E-Cho, F-Cho, HDL-Cho and LDL-Cho levels in control rats and rats with RAL. ■, Control rats; ▨, rats with RAL. Values are the mean ± SD. Significantly different from control rats: *$p<0.05$, **$p<0.01$.

the control group and at the 66th week in the RAL group. The longest surviving rat in the RAL group expired at 105 weeks, suggesting a shortened life span due to the RAL.

The remaining right kidney was normal at the time of the left renal artery ligation. However, BUN and Crn concentrations in the RAL group were higher than the control group by the 4th week. The renal artery ligation didn't result in a compensatory response from the contralateral kidney.

A report on cholesterol turnover and metabolism showed apparent decreases in old animals[12]. In aging rats, sterosclerosis and hyperlipidemia develop due to a rise in triglycerides and other lipoprotein fractions.

The present study showed that T-Cho, E-Cho and F-Cho concentrations in the control group became elevated with aging. In addition, they were higher in the RAL group than in the control group under an *ad libitum* feeding regime .

Bagdade et al.[10,11,13] showed that uremic patients often have elevated serum triglyceride levels. The present study showed that both total cholesterol and triglyceride levels were elevated at the 48th week after renal artery ligation. Renal artery ligation also resulted in hypertriglyceridemia.

It has been reported that hyperlipidemia and hypertriglycedemia may result from chronic renal failure[14,15]. In the present study, BUN, Crn, T-Cho, E-Cho and F-Cho concentrations were elevated after four weeks. This suggested that hyperlipidemia and hypertryglyceridemia result from uremic increases, or uremica results from hyperlipidemia after renal artery ligation when rats are fed *ad libitum.*

The daily food intakes in the RAL group were more than the control group after the 4th week, when the rats were fed *ad libitum.* However, the reasons for this are unclear.

A previous study showed that in old rats, hypercholesterolemia resulted from elevated LDL-and HDL-cholesterol levels, whereas elevated plasma concentrations of apolipoproteins B and E were attributed to elevated LDL-cholesterol and HDL-cholesterol concentrations[16].

In both the RAL and control groups of the present study, T-Cho concentration gradually increased

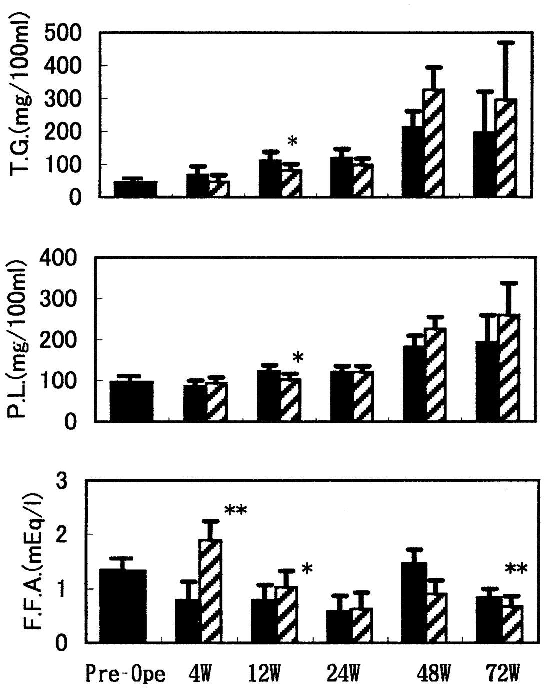

Fig. 4 Changes of serum TG, PL and FFA levels in control rats and rats with RAL. ■, Control rats; ▨, rats with RAL. Values are the mean ± SD. Significantly different from control rats: *p<0.05, **p<0.01.

with age. However, HDL-cholesterol concentrations were unchanged and LDL-cholesterol concentrations gradually increased in the control group. HDL-cholesterol concentrations of the RAL group were higher and LDL-cholesterol concentrations lower than the control group after the 4th week.

One study showed that food restriction retards functional activity, which supports the hypothesis that a mechanism in food restriction increases the life span of rats[17]. Restriction delays the onset and changes the chronological course of physiologic decline. Food restriction markedly delays the age-related loss of the lipolytic action of glucagon on adipocytes. Unlike glucagon, epinephrine is regarded as an important physiologic regulator of fat mobilization. Adipocyte function is not the only example of food restriction delaying age-related physiologic change. It is necessary to examine the influence on growth and life span in the rat RAL model under restrictive feeding conditions and diets of various compositions, since protein restriction was reported to damage renal function, as does amino acid fortification[18-21]. A low-calorie diet has also been studied in cats with renal failure[22]. In a previous study by the authors, RAL rats were arbitrarily divided into three groups according to post operative longevity: 38-58 weeks (average life span; 49.3 weeks), 60-72 weeks (average life span 66.1 weeks), and 74-104 weeks (average life span 86.3). It was found that the rats with the shortest life span gained weight more rapidly until the 25th week and those with the longest life span showed a shallow growth curve. The control group gained weight the most slowly[9]. It was concluded that it is necessary for RAL rats to control weight. In a rat with RAL, decreasing food intake and thus slowing growth, may be a primary factor in prolonging the life span. In addition, high blood pressure is considered to be a possible factor in shortening the lifespan as there are reports that RAL may cause high blood pressure, although blood pressure was not measured in the present study[2-7]. A rapid increase in food intake was unlikely, however, since there was no increase in BW in the RAL rats after 48 weeks. Catabolism of body protein also cannot be confirmed. It is necessary to examine growth and life span in RAL rats under restrictive feeding conditions and diets with protein and fat restriction.

The present study showed that RAL rats exhibited hyperlipidemia and renal failure. We previously reported that the remaining kidney of rats in the RAL group, weighed three to four times more than controls at the 76th week after surgery[9]. Several azotemic factors such as arterial calcification and tissue necrosis have been reported[23-25]. If arterial calcification is pathogenically significant, it may be important to maintain serum phosphate concentrations close to normal[14].

There have been few studies on the nutrition and dietary habits of persons living with one kidney as a result of accidents or transplants. Therefore, the renal artery was ligated in rats to simulate a one-kidney model and metabolic changes were evaluated. The rats, maintained with free access to food, showed decreases in BUN and Crn as parameters of renal function, and also increases in T-Cho and E-Cho accompanied by a decrease in HDL-Cho 4 weeks after surgery. Hypertriglyceridemia and high density-lipoproteinemia are considered to be lipid abnormalities characteristic of renal failure[26]. These results suggest that protein and lipid metabolic abnormalities appear immediately after surgery in RAL rats under unrestricted feeding conditions.

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