Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: https://www.tandfonline.com/loi/irnf20

Evaluation of Two Protocols of Uremic Rat Model: Partial Nephrectomy and Infarction

Zun Chang Liu, Kai Ming Chow & Prof. Thomas Ming-Swi Chang

To cite this article: Zun Chang Liu, Kai Ming Chow & Prof. Thomas Ming-Swi Chang (2003) Evaluation of Two Protocols of Uremic Rat Model: Partial Nephrectomy and Infarction, Renal Failure, 25:6, 935-943, DOI: 10.1081/JDI-120026028

To link to this article: https://doi.org/10.1081/JDI-120026028

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

RENAL FAILURE
Vol. 25, No. 6, pp. 935–943, 2003

LABORATORY STUDY

Evaluation of Two Protocols of Uremic Rat Model: Partial Nephrectomy and Infarction

Zun Chang Liu, M.D.,[1] Kai Ming Chow, M.R.C.P.,[1,2] and Thomas Ming-Swi Chang, O.C., M.D., C.M., Ph.D., F.R.C.P.(C), F.R.S.C.[1,*]

[1]Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, Montreal, Quebec, Canada
[2]Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

ABSTRACT

Animal models of chronic renal failure have been mostly achieved by partial ablation of renal parenchyma, the two most common techniques employed being surgical resection or infarction. Evaluation of the uremic model using these two techniques was carried out in Wistar rats. Two weeks after operative procedure, measured serum urea levels in the resection and infarction models were 59.1 and 64.3 mg/dL (normal range 15.6–24.4 mg/dL) respectively. However, the standard deviation in the former was significantly lower, 6.3 vs. 97.1 mg/dL from infarction model, $p = 0.007$. A consistent degree of glomerular filtration rate reduction was obtained in the resection model, resulting in 20–30% of normal creatinine clearance. This compared favorably with the creatinine clearance range (0.3–74% of normal) from the infarction model, in which two animals died of uremia and seven had higher than 50% of normal creatinine

*Correspondence: Prof. Thomas Ming-Swi Chang, Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, Montreal, Quebec, H3G 1Y6 Canada; Fax: +1 (514) 3984983; E-mail: artcell.med@mcgill.ca.

DOI: 10.1081/JDI-120026028
0886-022X (Print); 1525-6049 (Online)

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

clearance. It is reasonable to attribute reproducibility and homogeneity demonstrated in the resection model to (i) more precise control of renal ablation extent with surgical techniques and (ii) less interplay of confounding injury mechanism to remnant kidney. These data support superiority of the resection model as an experimental tool for pathophysiological and/or interventional investigations of chronic renal failure.

Key Words: 5/6 Nephrectomy; Partial nephrectomy; Rat; Resection; Infarction; Uremic model.

INTRODUCTION

Animal models, the mainstay of experimental means to study chronic renal failure, have created the possibility of high-throughput therapeutic testing in laboratories. The prerequisite of developing a renal failure model is to define a target range of renal insufficiency so as to provide a stable uremic milieu allowing experimental manipulation. To date, the five-sixth nephrectomy model has remained state-of-the-art prototype although different ways to achieve five-sixth reduction of nephron mass have been proposed and practiced.[1–3] In principle, the model involves substantial removal of renal mass or nephron number, followed by compensatory renal hypertrophy in the remnant kidney.[4–7] The glomerular changes, in proportion to the amount of nephrons damaged, are characterized by hyperperfusion and hyperfiltration. Increasing workload by the remaining pool of nephrons characterizes the progression of renal injury, namely chronic renal failure. A steady decrease in the glomerular filtration rate is expected thereafter.

Nephrectomy or renal ablation as such has been most often achieved by means of either resection or infarction. In the former technique (resection model), parts of one kidney are resected, followed by contralateral nephrectomy.[3,8–10] The infarction model involves ligation of the renal artery from one kidney and contralateral nephrectomy.[1,11] Both techniques are described in a variety of animal species. Ideally, these techniques should induce a reproducible degree of glomerular filtration rate. Diminution of renal mass by different methods, however, might represent different degrees of renal injury and thus stages of chronic renal failure. This article describes our attempt to evaluate these two experimental models of chronic renal failure in rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rat (Charles River Laboratories) weighing 200–250 g were used. The rats were fed standard rodent chow containing 22.0% protein ad libitum.

Protocol I: Resection Model

The rats were anesthetized with pentobarbital (4 mg/100 g body weight intraperitoneally). With the animal in ventral recumbent position, a 2–3 cm skin incision was made caudal to the rib cage on the left side. After further muscle incision, the left kidney was dissected from the overlying fat and adrenal gland gently. A single silk thread was placed around the renal pedicle. The upper and lower kidney poles were excised after lifting the thread (in order to temporarily occlude the vascular flow during cutting). Meticulous resection was made under direct vision to ensure the remnant kidney represent 25% of intact kidney. With the remnant kidney wrapped in hemostatic gauze, it was returned into the abdominal cavity. Muscle wall was then sutured. Average time from the first cut to closure of the skin was 5 min.

One week after the first surgical procedure, right uninephrectomy was performed.

Protocol II: Vascular Ligation (Infarction Model)

Briefly, the left kidney was exposed and decapsulated in a similar manner as above. Instead of surgical ablation of renal tissue, the left renal arterial branches were isolated with blunt forceps and ligated using sterile silk suture. Functional nephrectomy was thus achieved by renal tissue infarction. Discoloration of 75% of the kidney was interpreted as ischemia that would progress to infarction. From the first cut to skin closure this procedure lasted 10 min. Right unilateral nephrectomy was then performed as in Protocol I.

Biochemistry

After completion of the five-sixth nephrectomy, longitudinal assessment of body weight, laboratory data including serum urea, creatinine, urinary creatinine clearance, and fecal nitrogen excretion was recorded. Creatinine clearance was estimated from consecutive timed urine collection using metabolic cages, each lasting 24 h. Results were expressed as mean $\pm$ SD unless otherwise stated. All statistical tests were performed with the SPSS for Windows version 11.0 statistical package (SPSS Inc., Chicago, IL). Statistical significance was accepted at the level of $p < 0.05$ (two-sided) for all analysis.

RESULTS

Twelve Wistar rats underwent five-sixth nephrectomy by surgical resection (Protocol I); another 14 rats had partial nephrectomy by infarction (Protocol II). Preoperatively, serum levels of urea in two groups were 17.7 ± 3.0 mg/dL (Protocol I) and 15.9 ± 3.8 mg/dL (Protocol II) respectively. No surgical mortality occurred.

After an abrupt weight loss within the first week of surgical procedure, all animals exhibited an increase in body weight thereafter, doubling the baseline body weight

after 8 weeks (Fig. 1). Serum urea and creatinine concentrations stabilized two weeks after the operation. On average, measured serum urea level rose to 59.1 mg/dL and 64.3 mg/dL (normal range 15.6–24.4 mg/dL) respectively at two weeks in two protocols. Degree of uremia among individual animals in infarction model (Protocol II) was markedly variable. The standard deviation of serum urea concentration was 97.1 mg/dL from infarction model, far in excess of the figure of 6.3 mg/dL from resection model ($p = 0.007$) (Fig. 2). Creatinine clearance of rats from resection (Protocol I) and infarction (Protocol II) models measured 0.28 ± 0.08 mL/min/100 g body weight and 0.46 ± 0.17 mL/min/100 g body weight respectively at two weeks (normal range 0.75–1.01 mL/100 g body weight). In both protocols, the creatinine clearance was decreased, but the standard deviation among the individuals of Protocol II was far in excess of the Protocol I (Fig. 3).

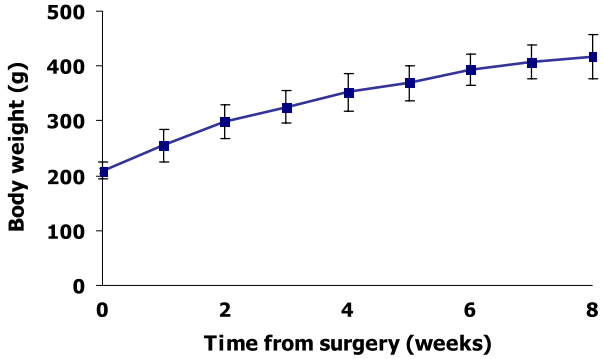

Figure 1. Time course of body weight of rats for the resection model.

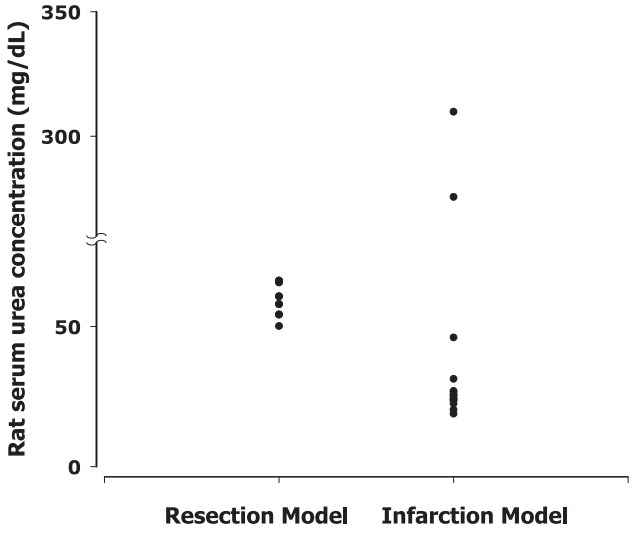

Figure 2. Significant inter-individual variation of uremia from infarction model, as reflected by rat serum urea concentration at two weeks after surgery.

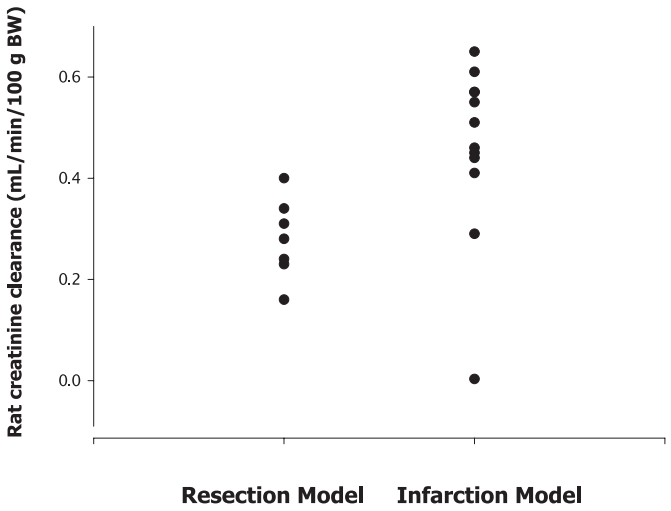

Figure 3. Significant inter-individual variation of uremia from infarction model, as reflected by measured creatinine clearance at two weeks after surgery.

Estimated creatinine clearance in one half of the rats from infarction model (Protocol II) remained greater than 50% of normal. Two rats from infarction model, at the other extreme, died during the observation period, both of which had serum urea level above 90 mg/dL before death. In the resection model (Protocol I), the plasma urea and creatinine clearance maintained the stable decreased levels during the observation period (Figs. 4 and 5).

Rats from the resection model give rise to a homogenous group of uremic animals with relatively stable biochemical and growth parameters. Table 1 shows the panel of laboratory data 8 weeks after the five-sixth nephrectomy. Serum urea concentration was maintained in the range of 55–65 mg/dL throughout the 8 weeks. Progression of underlying renal disease was reflected by a modest and steady decline of creatinine clearance, from 0.28 ± 0.08 mL/min/100 g body weight at second week to 0.20 ± 0.09 mL/min/100 g body weight after 8 weeks. This represents 20–30% of normal creatinine clearance. No significant variations of blood electrolytes and total protein were observed at week 8 post operation.

DISCUSSION

A variety of procedures for inducing uremia have been reported in literature,[12–15] but little standardized methodology exists. Consistency of nephron mass reduction, and thus reaching the desired degree of uremia, has been difficult because of failure to estimate the renal injury intra-operatively. Intuitively, the criterion to evaluate animal model of uremia is a reproducible degree of renal insufficiency. A homogenous experimental animal model with quantitatively uniform progression of renal injury, as reflected by our longitudinal assessment of serum urea, urinary creatinine clearance and growth rate, was herein presented after

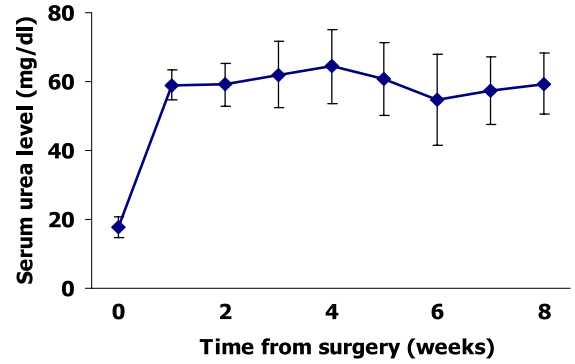

Figure 4. Time course of rat serum urea concentration for the resection model.

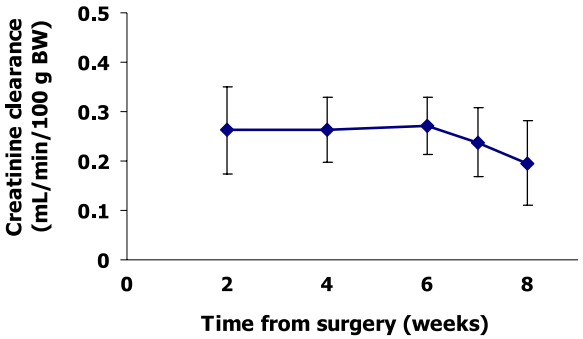

Figure 5. Time course of rat creatinine clearance for the resection model. Normal reference value of creatinine clearance in rats is 0.75–1.01 mL/min/100 g body weight.

Table 1. Laboratory parameters of uremic rats after 8 weeks in the resection model.

	Uremic rats	Reference value
Serum chloride (mmol/L)	120 ± 3.5	100–106
Serum sodium (mmol/L)	157.2 ± 4.6	144–154
Serum potassium (mmol/L)	6.3 ± 0.7	5.4–7.7
Serum calcium (mmol/L)	3.3 ± 0.1	2.7–3.2
Serum phosphate (mmol/L)	3.12 ± 0.36	1.38–2.85
Serum albumin (g/L)	41.2 ± 3.7	28–44
Serum total protein (g/L)	70.6 ± 2.3	64–84
Serum uric acid (μmol/L)	36.2 ± 15.7	53–112
Serum urea (mg/dL)	59.4 ± 8.9	15.6–24.4
Creatinine clearance (mL/min/100 g BW)	0.20 ± 0.09	0.75–1.01
Fecal nitrogen (mg/24 h/100 g BW)	40.9 ± 7.4	43.1–57.4

surgically induced five-sixth nephrectomy. Furthermore, the current study confirms the reliability of surgical resection model of inducing experimental chronic renal failure in rats, with satisfactory inter-individual variability of uremia as opposed to the strikingly wide variation observed in the infarction model.

Ormrod and Miller used surgical technique, with precise determination of the remnant renal tissue amount, to achieve standardized five-sixth nephrectomy.[16] Minimizing the error margin in assessing the residual renal mass was central to induce the predetermined degree of uremia. Our experience and data herein described concurs with the literature that failure to appreciate the extent of compensatory growth in the remaining renal tissue was primarily the reason of variable azotemia achieved.[17–19] In case of infarction model, distinction between devascularized tissues and normal tissues is less clear-cut and therefore the amount of intact nephrons to be left behind would be much more difficult to control. The same uncertainty applies to the use of electrocautery or cryosurgery for renal ablation.[12,13] In keeping with our findings, unpredictable serum urea levels had been unanimously reported in studies comparing the resection with ligation techniques.[20,21] Only two out of seven dogs, for instance, demonstrated the desired level of azotemia as achieved by ligation methods whereas half of the animals had more than 50% of normal clearance.[21] Of note, these results in large-sized animals closely resembled our finding in rat models by infarction technique.

Although our analysis was hampered by the absence of postmortem examination, findings from canine model demonstrated the formation of collateral vessels bypassing the ligated branches after vascular infarction.[20] Besides, ramification pattern of renal artery varies greatly from one animal to animal, thus compromising the efforts to standardize extent of nephron injury. The infarction model is further confounded by additional element of renovascular hypertension owing to compromised blood supply.[22] More recently, additional injury mechanism by virtue of infarction model has been reaffirmed. Such evidence comes from the studies comparing surgical resection model with infarction model (in rats).[23–25] With equivalent reduction of renal mass, uremic animals in the infarction model demonstrated significant proteinuria, hypertension, and glomerulosclerosis, which were not seen in resection model. Glomerular capillary hypertension, an important harbinger of progressive renal injury,[26] was also considerably higher in the case of infarction model.[24] With these caveats and support from our data, we believe that infarction model should not as yet replace the contemporary and time-honored five-sixth nephrectomy model created by surgical resection.

REFERENCES

1. Gretz, N.; Waldherr, R.; Strauch, M. The remnant kidney model. In *Experimental and Genetic Rat Models of Chronic Renal Failure*; Gretz, N., Strauch, M., Eds.; Karger: Basel, 1993; 1–28.
2. Strauch, M.; Gretz, N. Animal models to induce renal failure: a historical survey. Contrib. Nephrol. **1988**, *60*, 1–8.
3. Morrison, A.B. Experimentally induced chronic renal insufficiency in the rat. Lab. Invest. **1962**, *11*, 321–332.

4. Fine, L. The biology of renal hypertrophy. Kidney Int. **1986**, *29*, 619–634.
5. Malt, R.A. Compensatory growth of the kidney. N. Engl. J. Med. **1969**, *280*, 1446–1459.
6. Hostetter, T.H.; Olson, J.L.; Rennke, H.G.; Venkatachalam, M.A.; Brenner, B.M. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. J. Am. Soc. Nephrol. **2001**, *12*, 1315–1325.
7. Peters, G. Introduction: history and problems of compensatory adaptation of renal functions and of compensatory hypertrophy of the kidney. Yale J. Biol. Med. **1978**, *51*, 235–245.
8. Chanutin, A.; Ferris, E.B. Experimental renal insufficiency produced by partial nephrectomy. Arch. Intern. Med. **1932**, *49*, 767–787.
9. Platt, R.; Roscoe, M.H.; Wemyss-Smith, F. Experimental renal failure. Clin. Sci. **1952**, *11*, 217–231.
10. Waynforth, H.B.; Flecknell, P.A. Nephrectomy, In *Experimental and Surgical Technique in the Rat*, 2nd Ed.; Waynforth, H.B., Flecknell, P.A., Eds.; Academic Press: London, 1992; 274–275.
11. Avioli, L.V.; Scott, S.; Lee, S.W. Intestinal calcium absorption: nature of defect in chronic renal disease. Science **1969**, *166*, 1154–1156.
12. Boudet, J.; Man, N.K.; Pils, P.; Sausse, A.; Funck-Brentano, J.L. Experimental chronic renal failure in the rat by electrocoagulation of the renal cortex. Kidney Int. **1978**, *14*, 82–86.
13. Kumano, K.; Kogure, K.; Tanaka, T.; Sakai, T. A new method of inducing experimental chronic renal failure by cryosurgery. Kidney Int. **1986**, *30*, 433–436.
14. Okuda, S.; Oh, Y.; Tsuruda, H.; Onoyama, K.; Fujimi, S.; Fujishima, M. Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. Kidney Int. **1986**, *29*, 502–510.
15. Fukuda, S.; Kopple, J.D. Chronic uremia syndrome in dogs induced with uranyl nitrate. Nephron **1980**, *25*, 139–143.
16. Ormrod, D.; Miller, T. Experimental uremia: description of a model producing varying degrees of stable uremia. Nephron **1980**, *26*, 249–254.
17. Prakash, S.; Chang, T.M. Microencapsulated genetically engineered live *E. coli* DH5 cells administered orally to maintain normal plasma urea level in uremic rats. Nat. Med. **1996**, *2*, 883–887.
18. Prakash, S.; Chang, T.M. In vitro and in vivo uric acid lowering by artificial cells containing microencapsulated genetically engineered *E. coli* DH5 cells. Int. J. Artif. Organs **2000**, *23*, 429–435.
19. Prakash, S.; Chang, T.M. Artificial cells microencapsulated genetically engineered *E. coli* DH5 cells for the lowering of plasma creatinine in-vitro and in-vivo. Artif. Cells Blood Substit. Immobil. Biotechnol. **2000**, *28*, 397–408.
20. Vaneerdeweg, W.; Buyssens, N.; De Winne, T.; Sebrechts, M.; Babloyan, A.; Arakelian, S.; De Broe, M.E. A standardized surgical technique to obtain a stable and reproducible chronic renal failure model in dogs. Eur. Surg. Res. **1992**, *24*, 273–282.
21. Brassard, J.A.; Meyers, K.M.; Person, M.; Dhein, C.R. Experimentally induced renal failure in the dog as an animal model of bleeding. J. Lab. Clin. Med. **1994**, *124*, 48–54.

22. Gretz, N.; Meisinger, E.; Strauch, M. Partial nephrectomy and chronic renal failure: the 'mature' rat model. Contrib. Nephrol. **1988**, *60*, 46–55.
23. Griffin, K.A.; Picken, M.; Bidani, A.K. Method of renal mass reduction is a critical modulator of subsequent hypertension and glomerular injury. J. Am. Soc. Nephrol. **1994**, *4*, 2023–2031.
24. Griffin, K.A.; Picken, M.M.; Churchill, M.; Churchill, P.; Bibani, A.K. Functional and structural correlates of glomerulosclerosis after renal mass reduction in the rat. J. Am. Soc. Nephrol. **2000**, *11*, 497–506.
25. Garber, S.L.; Mirochnik, Y.; Brecklin, C.; Slobodskoy, L.; Arruda, J.A.; Dunea, G. Effect of relaxin in two models of renal mass reduction. Am. J. Nephrol. **2003**, *23*, 8–12.
26. Ofstad, J.; Horvei, G.; Kvam, F.I.; Mørkrid, L.; Sekse, I.; Svarstad, E.; Iversen, B.M. Glomerular hemodynamics in progressive renal disease. Kidney Int. Suppl. **1992**, *36*, S8–S14.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016