

An Established Rat Model of Inducing Reversible Acute Tubular Necrosis

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Introduction. Acute tubular necrosis (ATN) is a challenging problem that still requires to be studied in animal models. Our aim was to prepare an established experimental model of inducing reversible ATN in rats by determining the optimum duration of ischemia induction to the kidney.

Materials and Methods. Twenty-four hour after nephrectomy of the right kidney and clamping the pedicle of the left kidney for durations ranging from 10 to 55 minutes, the kidney function and the histologic changes were evaluated. Accordingly, the optimum duration of clamping was determined and in the next step, it was considered for induction of reversible ATN in another group of rats. This group was followed up for 14 days and the pathologic course and function of the kidney were observed.

Results. Reversible ATN developed by 47-minute clamping of the renal pedicle. Blood urea nitrogen and serum creatinine levels were elevated up to threefold within 24 hours after the induction of ischemia and they decreased to their reference ranges after 12 and 6 days, respectively. In the histologic study of the kidneys, the least extend of injury was noted by the 14th day following the ATN induction. Even on the 14th day of the follow-up, some signs of ATN remained indicating that the tissue regeneration was not complete yet.

Conclusions. To integrate the experimental models of ATN, a rat model with 47-minute clamping of the renal pedicle for induction of ischemia seems appropriate. The resultant ATN remains for a long duration, while kidney function is alleviated.

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INTRODUCTION

Acute tubular necrosis (ATN) is a common affliction of the kidney with high mortality and morbidity rates that can lead to end-stage renal disease (ESRD) and reduced patient survival rate.^{1,2} The management of ATN is challenging and its treatment is still conservative, based on judicious fluid management, food supplements, and hemodialysis.³ So far, no optimal therapeutic intervention has been offered.⁴ These facts have

encouraged lots of researches on ATN which are mainly focused on the pathophysiology and therapeutic interventions that may prevent from ATN or reduce its severity and duration.⁵ The majority of such studies are being done on animal models. The advantages of using animal models compared to the studies on humans are: greater potential for manipulation, easier isolation of variables, less complexity, better understanding of the mechanisms, and less experimental limitations.^{5,6}

In their investigation on ATN, many scientists have found it convenient to study on rat and mouse models because of their advantages on other animals. Acute tubular necrosis in these models is mainly induced by complete occlusion of the renal artery or pedicle for various time periods.^{6,7} However, a standard period for achieving ATN in rat models has not been proposed yet. The aim of our study was to find the ideal duration of complete occlusion of the renal pedicle in a rat model of reversible ischemic tubular damage and monitor its effect on the function and histology of the kidney.

MATERIALS AND METHODS

The experiments were performed on congenic inbred male Wistar rats weighing 220 g to 250 g. Since susceptibility to ischemia is different between male and female rats,⁵ only male rats were used in the study. The animal procedure was approved and conducted in conformity with institutional guidelines and national laws. To determine the ideal duration of the renal pedicle occlusion for a reversible model of ATN, the right kidneys of 7 rats were nephrectomized and the pedicle of the left kidneys were clamped for 10, 20, 30, 40, 45, 50, and 55 minutes (each of these time periods was tried in 1 rat). Serum creatinine levels and histology of the left kidney were evaluated 24 hours after the operation in a blind fashion for signs of the ATN. The ideal duration for occlusion was defined as the longest duration of the occlusion of renal pedicle that made elevation in serum creatinine level and signs of ATN in histology without extensive necrosis. If necessary, the procedure was repeated on another rat to examine the times between the above-scheduled durations.

To induce the ATN, the rats were anesthetized by intramuscular injection of ketamine and promazine. The kidneys were exposed by a midline abdominal incision. Because of a longer renal pedicle, the left kidney was chosen to be clamped. The right kidney was nephrectomized and the pedicle of the left kidney was clamped by nontraumatic microvascular clamp for the scheduled time periods. Reperfusion was visualized directly after removal of the clamp. All of the operations were carried out by a single surgeon who gained enough experience in surgery on rats' kidneys before the study. The rats that had uncontrolled bleeding or

trauma to the organs during the operation and the ones in which reperfusion was not visualized were excluded from the study. As a control group, 2 rats underwent nephrectomy of the right kidney and the incision was repaired without occlusion of the renal pedicle.

In the next step, 10 other rats underwent a same procedure and followed for a 14 days' period after inducing reversible ATN by clamping of the renal pedicle for determined ideal duration. Blood urea nitrogen and serum levels were examined twice in 2 blood samples for each rat, and then, they were sacrificed on different days during the follow-up period and the kidneys were sent to a proficient pathologist. For evaluating the histology, the kidneys were fixed in formalin and embedded in paraffin. Four-micrometer sections were stained with hematoxylin-eosin, periodic acid-Schiff, and trichrome. The sections were evaluated blindly and the histopathology of all the kidneys was scored per section in at least 10 randomly selected nonoverlapping fields at $\times 400$ magnification. The results were scored as the percentage of damaged tubules: 0, no damage; 1, area of tubular damage less than 25%; 2, damage of 25% to 50%; and 3, damage of more than 50%. The loss of brush borders from proximal tubules, tubular cell swelling, interstitial edema, tubular dilation, luminal hyaline casts, and specially the extent of epithelial cell necrosis were used as evidence of tubular damage (Figure 1). The collected data were used to investigate changes in kidney function and ATN during the follow-up.

RESULTS

In the first part of the study, we clamped the left renal pedicle for the periods of time that varied from 10 to 55 minutes. In the 2 control rats without clamping of the renal pedicle, serum creatinine level was within the reference range (median, 0.7 mg/dL). The results of the serum creatinine levels and histology of the kidney in the rats with clamping are shown in Figure 2 and Table 1. After clamping the pedicle of the left kidney for 45 minutes, we observed a significant elevation of serum creatinine level and grade 2 ATN on pathology examination. A 50- and 55-minute occlusion of the renal pedicle resulted in extensive necrosis in the kidney tissue. To find the optimum duration for inducing reversible ATN, we tried 47-minute

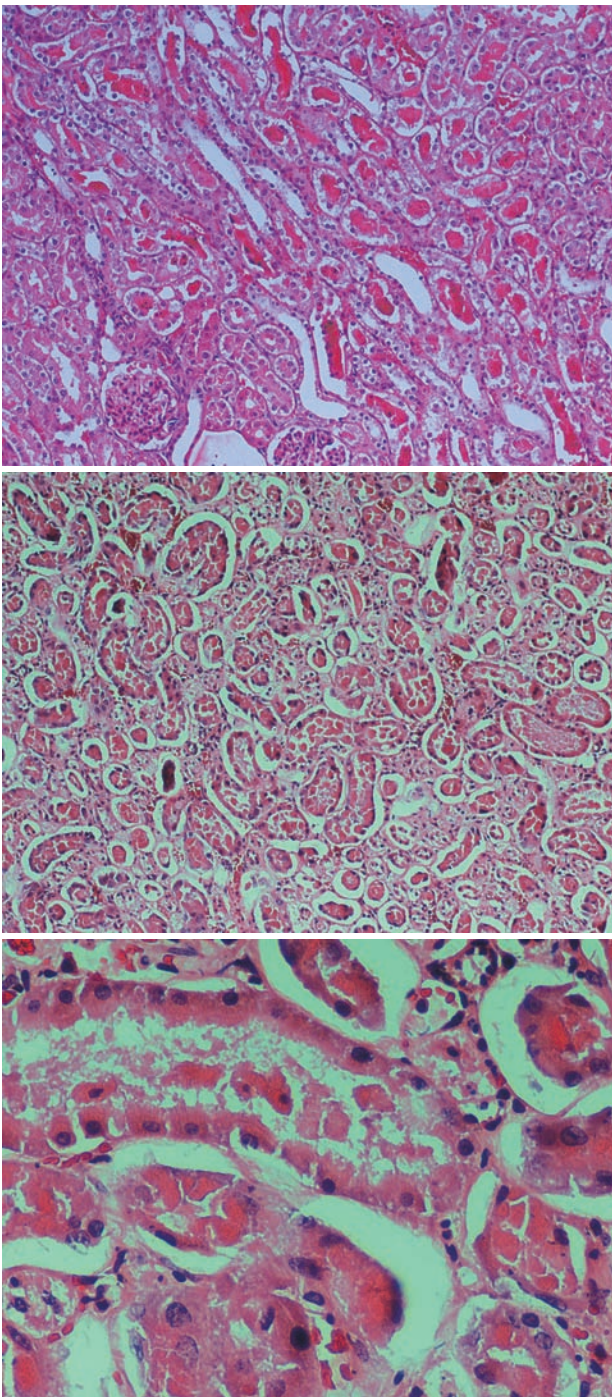


Figure 1. Pathologic changes in the kidney of rats with ischemia induced for achieving acute tubular necrosis. **Top**, tubular cells swelling and luminal hyaline casts (hematoxylin-eosin, × 200). **Middle**, Acute tubular necrosis of the epithelial cells and desquamation (hematoxylin-eosin, × 200). **Bottom**, Acute tubular necrosis of the epithelial cells and desquamation (hematoxylin-eosin, × 400).

clamping of the left renal pedicle in 2 other rats. On the first postoperative day, the median serum creatinine level was 2.6 mg/dL and the pathology

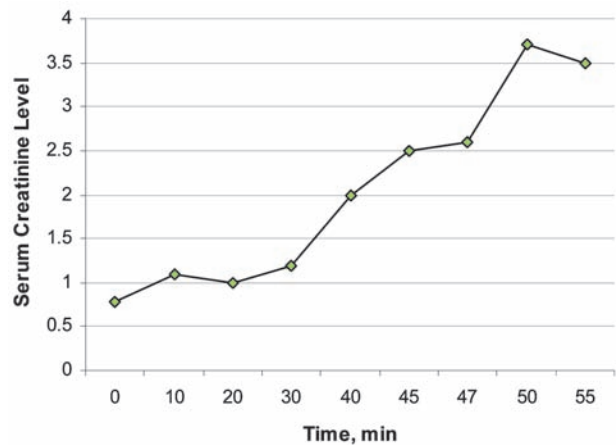


Figure 2. Serum levels of creatinine 24 hours after each time period attempted for clamping of the renal pedicle to induce ischemia/reperfusion in rats.

Table 1. Kidney Histology in Each Time Period of Renal Pedicle Occlusion 24 Hours After Ischemia/Reperfusion in Rats

Duration of Renal Pedicle Occlusion, min	Histologic Grade*
10	0
20	0
30	1
40	1
45	2
47	3
50	Severe necrosis
55	Severe necrosis

*Grading of histology is described in the Materials and Methods.

examination showed grade 3 ATN.

In the second part of the study, we clamped the pedicle of the left kidney for 47 minutes in 10 rats. The kidney function and histology that were studied during the 2-week follow-up are shown in Table 2. Blood urea nitrogen and serum creatinine levels reached their reference ranges after 12 days and 6 days, respectively. Evaluation of the kidney tissue sections showed signs of ATN and necrosis during the 2 weeks' follow-up.

DISCUSSION

Acute tubular necrosis is a common problem which is the challenging subject in many studies. To investigate the pathophysiology of ATN, studying on animals, especially rat and mouse models, has many advantages. A well established model of reversible ATN is warranted to have a good basis for many of these studies, facilitating research, and preventing from bias in the research

Table 2. Kidney Function and Histology During a 2-week Follow-up After Clamping of Renal Pedicle in Rats for 47 Minutes*

Characteristics	Day											
	1	2	3	4	5	6	7	8	10	11	12	14
First rat												
Rat code	1	1	2	3	2	4	3	5	5	6	7	6
BUN, mg/dL	440	282	363	119	109	94	75	81	79	74	68	70
Creatinine, mg/dL	4.1	2.2	2.6	1.3	0.9	0.8	0.9	0.8	0.8	0.9	0.8	0.9
Histology	...	3	2	...	3	...	2	1
Second rat												
Rat code	8	8	9	10	9	...	10	4	7
BUN, mg/dL	173	108	98	119	93	...	92	94	60
Creatinine, mg/dL	1.7	1.1	1.1	1.1	0.8	...	0.8	0.7	0.7
Histology	...	3	2	...	2	2	2
Mean BUN, mg/dL	306	195	231	119	161	94	84	88	79	74	68	65
Mean creatinine, mg/dL	2.9	1.7	1.9	1.2	0.9	0.8	0.9	0.8	0.8	0.9	0.8	0.8

*Grading of histology is described in the Materials and Methods. BUN indicates blood urea creatinine. Ellipses indicate that no examination was done.

results. However, there is no suggested method as a standard approach for induction of ATN.

The duration of clamping the renal vessels for induction of ischemia is a matter of controversy. The optimum duration of renal pedicle occlusion for achieving reversible ATN was 47 minutes in our study, which is consistent with the results in most other studies.⁸⁻¹² This time period varied between 30 to 45 minutes in other mouse and rat models.⁷⁻¹² Sabbatini and colleagues achieved ATN by a 30-minute clamping of both renal arteries.⁷ In a study on the protective effect of molsidomine and L-arginine in ischemia-reperfusion induced injury in rats, Chander and associates preferred 45-minute clamping of the renal pedicles.⁸ In our model of reversible ATN (47-minute occlusion of the renal pedicle), the blood levels of urea and serum creatinine returned to their normal range on days 12 and 6, respectively. Also the histologic evaluation showed signs of ATN even after 14 days of follow-up, which shows that tissue regeneration and repair was not yet complete. These data confirm that our model of ATN could be a good model for studies, helping to better understand the pathophysiology of ATN and to find ideal interventions for prevention and treatment of ATN.

CONCLUSIONS

Our findings demonstrated that to integrate the results obtained from different experimental models of ATN, we can use rat model with 47-minute clamping of the renal pedicle for induction of ischemia. This results in ATN that remains for a relatively long duration, while the kidney function

is alleviated. Similar studies on experimental models of ATN are recommended.

CONFLICT OF INTEREST

None declared.

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