A rat model of contrast-induced acute kidney injury following intra-arterial contrast administration

Zhiqing Wang¹

¹Department of Cardiology, 900 Hospital of the Joint Logistics Team, Fujian Medical University, Fuzhou, China

Zhiqing Wang

DOI:

dx.doi.org/10.17504/protocol.s.io.472gzqe

Protocol Citation: Zhiqing Wang . A rat model of contrast-induced acute kidney injury following intra-arterial contrast administration.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.472gzqe>

MANUSCRIPT CITATION:

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: Jul 06, 2019

Last Modified: Jul 06, 2019

PROTOCOL integer ID:
25562

Keywords: Contrast-induced acute kidney injury, rat model

ABSTRACT

Contrast-induced acute kidney injury (CI-AKI) is an iatrogenic complication frequently developed after cardiac catheterization procedures with the administration of [iodinated contrast medium](#). However, the precise pathological mechanisms remain obscure. Thus, the establishment of a reproducible rat model following [intra-arterial contrast medium \(CM\) injection](#) to simulate [the process of arteriography](#) is of great importance. Here, a detailed protocol for establishing a CI-AKI model was describe. Three-month-old male Sprague-Dawley rats, weighing approximately 300~400g at the start of the experiment, were used. The rats were pretreated with water dehydration for 48 hours, plus indomethacin and [N-ω nitro-L-arginine methyl ester](#) (L-NAME) injection, before intra-arterial contrast medium (CM) administration. Levels of serum creatinine (SCr) were measured and histopathological assessment of the kidney tissue by H&E staining were conducted. As expected, the administration of CM, following the pretreatment by indomethacin and L-NAME, induced remarkable renal dysfunction at 12 hours [post-procedure](#) in comparing with controls. The pronounced histopathological alterations in the tubular epithelial cells of the inner medulla and outer medulla were also observed in the CI-AKI rats. Thus, this rat model was reliable as a valuable tool for exploring the pathogenesis of CI-AKI.

MATERIALS

MATERIALS

indomethacin **Contributed by users**

N-Nitro-L-arginine Methyl Ester Hydrochloride **Contributed by users**

1 Surgical Procedure

1.1 Rats were deprived of water for 48 [hours](#). Body weight of each rat was measured.

- 1.2** Deeply anaesthetized was conducted by pentobarbital sodium (40 [mg/kg, i.p.](#))
- 1.3** The right femoral vein and carotid artery were both cannulated by short peripheral catheters which were widely used in the nursing practice (24 G×21 mm).
- 1.4** A baseline blood sample was drawn (1 ml) from the carotid artery for determination of SCr.
- 1.5** Indomethacin was dissolved in phosphate buffer (pH 8.4) at a concentration of 5mg/ml, then administrated intravenously at the dose of 10 mg/kg. After 15 min, L-NAME (dissolved in 0.9% normal saline at a concentration of 10mg/ml) was administrated intravenously at the dose of 10 mg/kg. After another 15 min, these rats were randomized to receive iopromide (CI-AKI group, n = 9) or normal saline (Control group, n = 9) at the dose of 7.8 ml/kg via the carotid artery cannulation over a time course of 5 min.
- 1.6** The rats were then allowed to recover in individual cages with free access to tap water and standard chow.

2 Sample Collection

- 2.1** At 12 hours post CM injection, the rats were deeply anaesthetized by pentobarbital sodium (40 mg/kg, i.p.).
- 2.2** A second set of arterial blood sample was obtained from the ventral aorta.
- 2.3** The blood cells were removed by perfusing with 0.9% normal saline through the bilateral renal arteries.

2.4 Both kidneys were harvested and bisected longitudinally. The right kidney, containing cortex and medulla, was fixed in 10 % neutral formalin liquid for [histological](#) analysis.

2.5 The rats were euthanized with an overdose of pentobarbital anesthesia (200 mg/kg, i.p.).

3 Measurement of SCr level

3.1 Blood samples were centrifuged within 2 hours at 1000 × g for 15 min.

3.2 The levels of SCr were measured at the hospital clinical laboratory by a standard method.

4 Histopathological assessment of the kidney tissue by hematoxylin and eosin (H&E) staining.

4.1 The [histological](#) samples were embedded in paraffin and sectioned at 3 to 4 µm thick.

4.2 Deparaffinize each slide with xylene (repeated 3 times, 2 min each).

4.3 Hydrate tissue sections with gradient ethanol (100% ethanol repeated 3 times, 1 min each; 80% ethanol for 1 min) to distilled water in a fume hood.

- 4.4** Incubate the slides with hematoxylin solution in a staining jar for 4 min, and then rinse with tap water for 2 min to remove the unbound dye from slides.
- 4.5** Transfer the slides to 0.1% hydrochloric acid in alcohol for 30 sec. Then rinse with tap water for 1 min.
- 4.6** Transfer the slides to 0.5% ammonia solution for 1 min until the background turns light blue. Then rinse with tap water for 1 min.
- 4.7** Transfer the slides to 80% ethanol for 1 min.
- 4.8** Incubate the slides with 1% eosin in a staining jar for 50 sec, and then rinse with tap water to remove the unbound dye from slides.
- 4.9** Dehydrate with 100% ethanol (repeated 3 times, 1 min each) and clear in xylene (repeated 3 times, 1 min each).
- 4.10** The slides were then mounted with resinous mounting medium and stored at room temperature.
- 4.11** After being visualized by [H&E staining](#), the slides of each sample were reviewed by [lightmicroscope](#).
- 4.12** Tubular injury was graded by the Paller score, a semi-quantitative evaluation. For each kidney, 100 tubules from 10 highmagnification (×200) fields of the medulla and the outer [medulla](#) were scored.
-

5 Results

Expected result

As expected, the administration of CM, following the pretreatment by indomethacin and L-NAME, induced remarkable renal dysfunction at 12 hours post-procedure. The average elevation of SCr was (59.9 ± 23.0) % over baseline in the CI-AKI rats, comparing to (-10.5 ± 17.1) % in the control rats injected with normal saline (Table 1, $p < 0.05$). [The pronounced histopathological alterations in the tubular epithelial cells of the inner medulla and outer medulla were observed in the CI-AKI rats, including vacuole-like denaturation, nucleus pycnosis and apoptotic body formation](#)(Fig 1A). Additionally, the mean Paller score for the contrast treated kidneys was 8.2 ± 0.9 , whereas that for controls was 3.6 ± 1.2 (Fig 1B, $p < 0.05$), indicating of a greater extent of tubular injury after the intervention of contrast.

Table 1. Baseline characteristics of experimental groups and the effects of contrast medium on changes in SCr elevation

Group	Body weight (g)	SCr ($\mu\text{mol/L}$)	Percent increment of SCr (%)	
Baseline	End			
Control (n = 9)	370.8 ± 28.3	53.3 ± 9.2	47.9 ± 13.1	-10.5 ± 17.1
CI-AKI (n = 9)	342.2 ± 36.1	43.9 ± 12.3	$69.5 \pm 18.7^*$	$59.9 \pm 23.0^*$

Data are mean $\pm$ SD. Statistical analysis was performed using the Student's t test. $*P < 0.05$ vs. control group.

Fig 1. Histopathological assessment of the kidney tissue of control rats and select CI-AKI rats by H&E staining. (A) Contrast medium treatment induced representative histopathological changes in the extrarenal medulla of H&E-stained kidney sections. (B) [Tubular injury was graded by the Paller score, a semi-quantitative evaluation. For each kidney, 100 tubules from 10 highmagnification \(\$\times 200\$ \) fields of the medulla and the outer medulla in rats were scored.](#) The average Paller score was much higher in CI-AKI rats than controls (8.2 ± 0.9 vs. 3.6 ± 1.2 , $P < 0.05$).

A

B