A rat model of contrast-induced acute kidney injury following intra-arterial contrast administration V.1

Jul 16, 2019

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.z6bf9an

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ABSTRACT

Contrast-induced acute kidney injury (CI-AKI) is an iatrogenic complication frequently developed after cardiac catheterization procedures with the administration of iodinatedcontrast media. 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 12hours 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.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0218574

MATERIALS

NAME	CATALOG #	VENDOR
Indomethacin	I7378-10G	Sigma-aldrich
N-ω nitro-L-arginine methyl ester	N5717-1G	Sigma-aldrich

MATERIALS TEXT

Indomethacin and N-ω nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Indomethacin, an inhibitor of the synthesis of prostaglandin,was dissolved in phosphate buffer (pH 8.4) at a concentration of 5mg/ml. Iopromide (Ultravist 370; 370 mg/ml iodine), a nonionic monomeric low-osmolar CM widely used in the clinical practice, was obtained from Bayer Co. (Leverkusen, Germany).

Surgical Procedure

1 Rats were deprived of water for 48 hours

2 Body weight of each rat was measure.

3 Deeply anaesthetized was conducted by pentobarbital sodium (40 mg/kg, i.p.).

4 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).

5 A baseline blood sample was drawn (1 ml) from the carotid artery for determination of SCr.

6 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.

7 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.

8 After another 15 min, these rats were randomized to receive iopromide (CI-AKI group) or normal saline (Control group) at the dose of 7.8 ml/kg via the carotid artery cannulation over a time course of 5 min.

9 The rats were then allowed to recover in individual cages with free access to tap water and standard chow.

Sample Collection

10 At 12 hours post CM injection, the rats were deeply anaesthetized by pentobarbital sodium (40 mg/kg, i.p.).

11 A second set of arterial blood sample was obtained from the ventral aorta.

12 The blood cells were removed by perfusing with 0.9% normal saline through the bilateral renal arteries.

13 Both kidneys were harvested and bisected longitudinally. The right kidney, containing cortex and medulla, was fixed in 10 % neutral formalin liquid for histological analysis.

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

Measurement of SCr level

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

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

Histopathological assessment of the kidney tissue by H&E staining

17 The histological samples were embedded in paraffin and sectioned at 3 to 4 μm thick.

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

19 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.

20 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.

21 Transfer the slides to 0.1% hydrochloric acid in alcohol for 30 sec. Then rinse with tap water for 1 min.

22 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.

23 Transfer the slides to 80% ethanol for 1 min.

24 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.

25 Dehydrate with 100% ethanol (repeated 3 times, 1 min each) and clear in xylene (repeated 3 times, 1 min each).

26 The slides were then mounted with resinous mounting medium and stored at room temperature.

27 After being visualized by hematoxylin and eosin (H&E) staining, the slides of each sample were reviewed by lightmicroscope.

28 FTubular 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.

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 (Fig 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 2A). 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 2B, $p < 0.05$), indicating of a greater extent of tubular injury after the intervention of contrast.

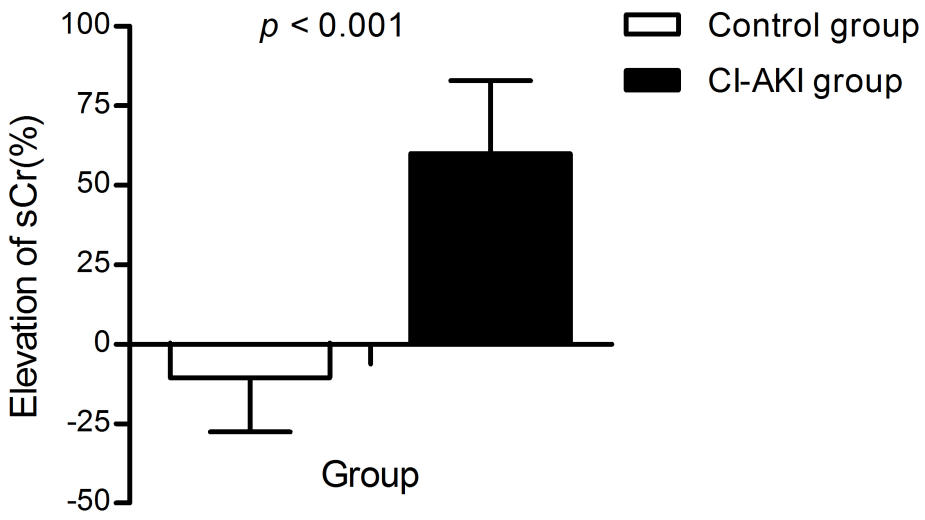

Fig 1.The averageelevation of sCr in CI-AKI rats was (59.9±23.0) % over baseline, comparedwith (-10.5±17.1) % in controls (p < 0.001).

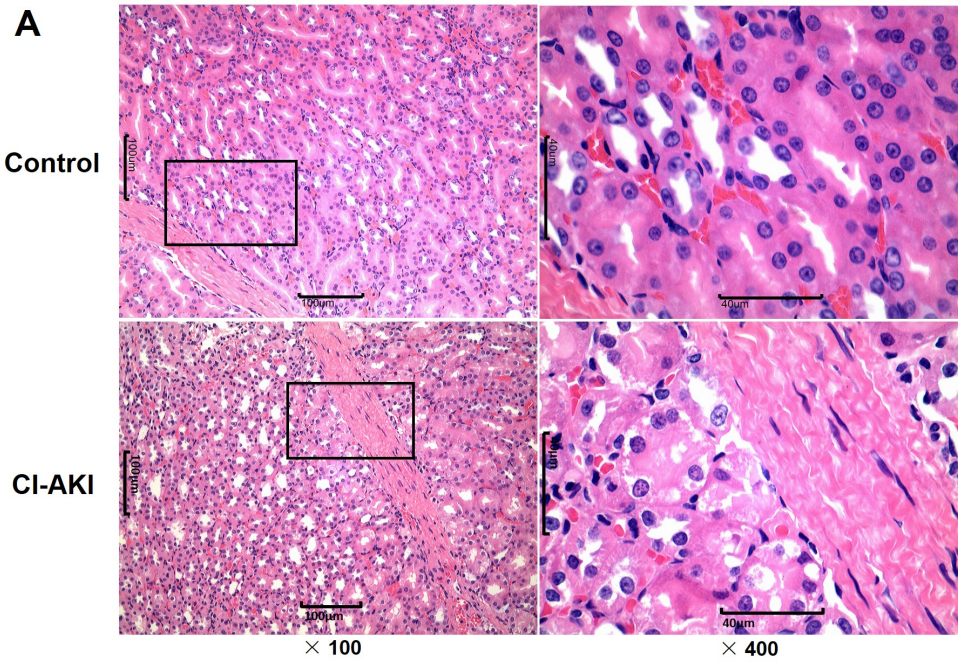

Fig 2A. Contrast medium treatment induced representative histopathological changes in the extrarenal medulla of H&E-stained kidney sections.

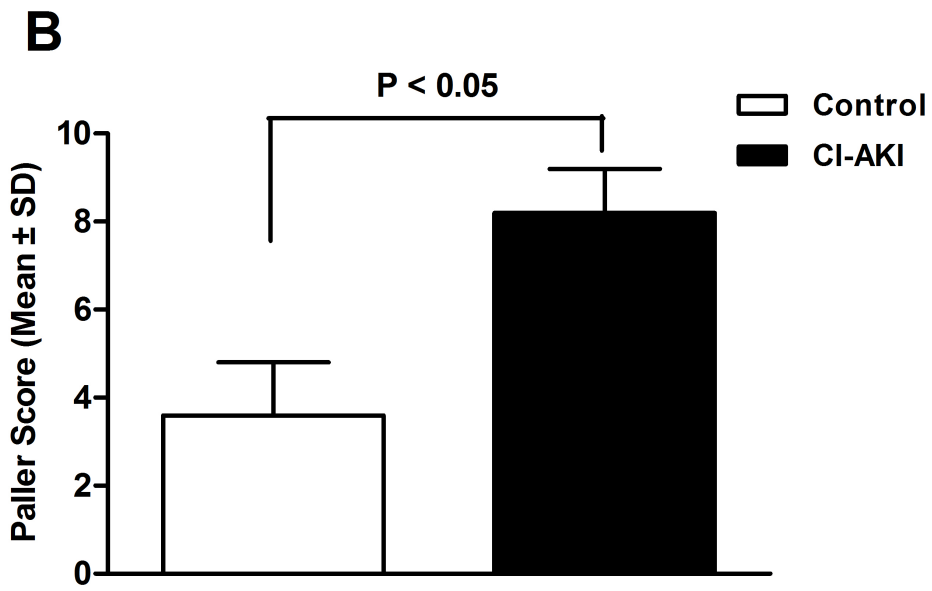

Fig 2B. The average Paller score was much higher in CI-AKI rats than controls (8.2 ± 0.9 vs. 3.6 ± 1.2, $P < 0.05$)