narra j

Short Communication

Effects of iopromide contrast agents on kidney iNOS expression and tubular histopathology alterations

Indrianto W. Pranoto[1,2], Tarmono Djojodimedjo[1,2*], Mohammad A. Soebadi[1,3] and Anny S. Rahaju[2,4]

[1]Department of Urology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; [2]Department of Urology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia; [3]Department of Urology, Universitas Airlangga Teaching Hospital, Surabaya, Indonesia; [4]Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: tar_urology@yahoo.com

Abstract

Contrast-induced acute kidney injury is a common complication marked by reduced kidney function within 48 hours of contrast administration. The aim of this study was to evaluate renal function, anatomy, and molecular changes at 24 hours, 48 hours, and 72 hours post-iodinated contrast media (ICM) administration. This true-experimental study used a post-test-only control group design. Rats underwent unilateral nephrectomy, followed by intravenous injection of ICM using iopromide 370 mg iodine/mL per rat at a dose of 231 mg iodine, and were then divided into four groups: control (C), rats terminated at 24 hours after iopromide administration (E24), rats terminated at 48 hours after iopromide administration (E48), and rats terminated at 72 hours (E72) after iopromide administration, with eight rats per group. Renal function (BUN and SCr levels) remained unchanged after 24, 48, and 72 hours of iopromide administration. Iopromide increased renal tubular damage, as shown by higher histopathological scores for loss of brush border and tubular necrosis, except for proteinaceous casts, where histopathological scores increase especially within the first 24 hours and decrease after 72 hours. Iopromide significantly altered iNOS expression in the glomerulus at 24 and 48 hours, and iNOS expression was decreased after 72 hours. iNOS expression in the intrarenal vascular and tubules was unaffected by iopromide administration. In conclusion, this study found no changes in renal function parameters, improvement in proteinaceous casts, and increased iNOS expression in the glomerulus, offering new insights into the effects of contrast on kidneys.

Keywords: Contrast-induced acute kidney injury, iopromide, renal function, histopathological examination, iNOS expression

Introduction

Contrast-induced acute kidney injury (CA-AKI) is a common complication with a high incidence and mortality rate that has not decreased over time [1]. CA-AKI refers to a decline in kidney function that occurs within 48 hours after administration of contrast material into the blood vessels [2]. The exact incidence of CA-AKI remains unclear, which may lead clinicians to underestimate CA-AKI [1]. One of the most common causes of CA-AKI is coronary angiography (CAG) [3].

Another study found a suspected incidence of CA-AKI in 3.1% of 32,308 patients undergoing ICM (Iodinated Contrast Media) examinations for cardiovascular organs [4]. It was noted that

https://narraj.org/

Received: July 24, 2024 - Accepted: October 10, 2024 - Published online: November 21, 2024

older age showed a tendency to develop CA-AKI more easily, especially in patients with comorbidities including chronic kidney disease, diabetes mellitus, acute heart failure, and cardiogenic shock [4]. Although CA-AKI is usually associated with iodine-containing contrast agents, it can also occur with non-iodine contrast agents such as gadolinium (GBCAs), and the risk is higher with contrast agents that have higher osmolarity [5]. There are various types of iodine contrast media, including iothalamate, ioxaglate, iodipamide, iopamidol, ioversol, ioxilan, iohexol, and iodixanol, all of which are classified as low-osmolar contrast media (LOCM), iso-osmolar contrast media (IOCM), or high osmolar contrast media (HOCM) [6]. One commonly used group of contrasts is LOCM, which includes iopamidol and ioxaglate, approved by the FDA in 1985, followed by ioversol in 1988, and iopromide in 2002 [1].

Markers commonly used to detect nephrotoxicity and kidney dysfunction are serum creatinine (SCr) and blood urea nitrogen (BUN), although they have limited sensitivity in identifying early kidney injury [7]. In humans, the clinical signs of Acute Kidney Injury (AKI) can be described according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines, which categorize the severity of AKI into three stages based on decreased urine output criteria that indicate early kidney damage [8]. Pathological signs of AKI in the tubules can include dilated tubular lumens, loss of brush border, and tubular epithelial flattening [9]. Additionally, signs of AKI can be identified by the formation of renal intratubular casts, such as proteinaceous casts and cellular intratubular casts [10].

Many markers can be used to detect inflammation in the kidneys, such as TNF-α, IL-1β, caspase-1, caspase-3, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [11]. Concerning the role of nitrous oxide (NO) in the inflammatory process, the enzymes related to the inflammatory process are inducible NOS, NOS2, and type II NOS [12].

In this study, an iopromide contrast agent was used to test its nephrotoxicity on rat models. Several markers used in this study include SCr and BUN. Histological parameters were also examined for the loss of brush border, tubular necrosis, and proteinaceous casts, along with immunohistochemistry parameters examining the expression of iNOS in the glomerulus, intrarenal vascular, and tubules. Therefore, the aim of this study was to investigate the effect of ICM by using iopromide administration on renal function test parameters, tubular histopathological changes, and iNOS expression in renal tissue.

Methods

Animals

Male *Rattus norvegicus*, Wistar strain, 4–12 weeks old (n=32; 100–200 g) were obtained from Biochemistry Laboratories, Medical Faculty, Universitas Airlangga. The rats were maintained in a room with each cage containing 2 rats at a temperature between 21 and 25°C with a 12-hour light-dark cycle. During the first 7 days of the acclimation period, the rats were given a standard diet.

Study design and sample size

This study was a true-experimental study using a post-test-only control group design. To assess the effect of ICM administration on renal function test parameters, tubular histopathological changes, and iNOS expression in renal tissue, animals were injected with contrast material (iopromide 370 mg Iodium/mL, Ultravist®, Bayer Healthcare, Berlin, Germany) per rat at 231 mg dose of iodine [13]. After the acclimatization phase, the rats underwent unilateral nephrectomy to ensure the effects of the contrast agent would be concentrated on one kidney. Anesthesia was administered before the nephrectomy using a ketamine/xylazine cocktail. The mixture contained ketamine at a dose of 91 mg/kg and xylazine at a dose of 9.1 mg/kg, administered intraperitoneally [14]. The rats were then divided into four groups: The first group (n=8) was a control group (C). The second group (n=8) was terminated after 24 hours of iopromide (E24), the third group (n=8 each group) was terminated after 48 hours of iopromide administration, and the fourth group (n=8 each group) was terminated after 72 hours of iopromide administration (E72). The study design in this research is presented in **Figure 1**.

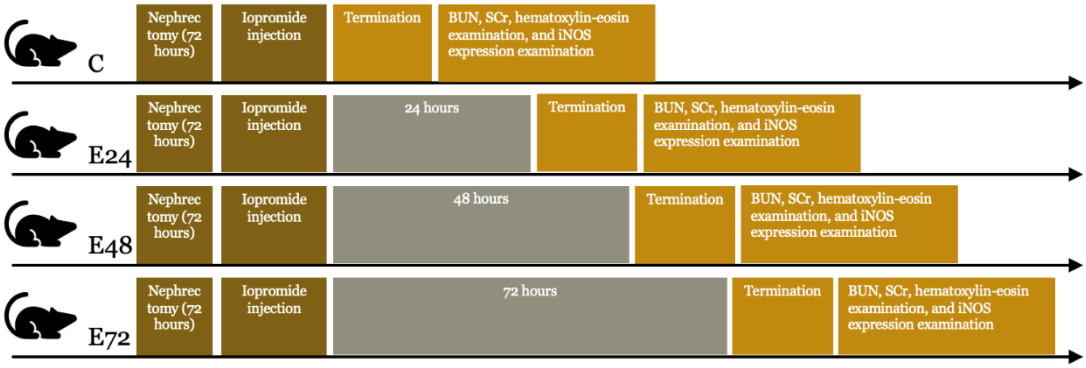

Figure 1. After acclimatization and randomization, the rats underwent a unilateral nephrectomy and were injected with iopromide. The rats were subsequently divided into four groups: the first group was a control group (C). The second group was terminated 24 hours after iopromide injection (E24), the third group was terminated 48 hours after iopromide injection (E48), and the fourth group was terminated 72 hours after iopromide injection (E72). After the rats were terminated, blood urea nitrogen (BUN) and serum creatinine (SCr) levels were measured, and histopathological examination and iNOS expression analysis were performed.

Randomization and blinding

The study employed simple randomization, randomly assigning all animals or samples to four distinct groups simultaneously without accounting for any additional variables. Blinding was used during the outcome assessments, including BUN, SCr, histopathological examinations, and immunohistochemistry examinations.

Endpoint

The administration of anesthetic drugs in this study adhered to the protocol outlined in the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia: 2020 Edition [15]. A combination of ketamine (300 mg/kg) and xylazine (30 mg/kg) was given via intraperitoneal injection. For animals weighing under 200 grams, cervical dislocation was performed while they were sedated, and decapitation was done using a guillotine on the sedated rats.

Blood urea nitrogen and serum creatinine test

Blood samples were taken from the heart ventricles (intracardial). The blood was analyzed for SCr using the modified Jaffe's kinetic method and for BUN using the enzymatic UV method.

Histopathological examination score

The examination of the renal tubular structure involved fixing kidney tissue in 10% formaldehyde for 24 hours. Subsequently, the tissue was stained with hematoxylin and eosin (H&E) to analyze changes in tubular structure, such as proteinaceous casts, tubular necrosis, and loss of the brush border. Tubular damage was assessed by examining ten fields of view for each section at 400× magnification.

An anatomical pathologist evaluated the findings using a previously established scale: 0 indicating no damage, 1 for mild damage (isolated, patchy unicellular damage), 2 for moderate damage (less than 25% damage), 3 for severe damage (25–50% damage), and 4 for very severe damage (more than 50% damage) [16].

Immunohistochemical examination

Bladder tissues were fixed in formalin and placed in paraffin blocks. Sections of 5 µm thickness were placed on slides and stained with antibodies: Anti-iNOS polyclonal antibody (#bs-2072R, Bioss). iNOS expression was observed using a light microscope at 400× magnification. The expression of iNOS was examined in the glomerular, intrarenal vascular, and renal tubules.

Scoring for iNOS expression was performed using the histochemical score (H-Score) method that combines staining intensity (i) and the percentage of cells stained at each intensity level (Pi). The intensity was assigned as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3

(strong staining). Pi values ranged from 0% to 100%. The H-Score was calculated using the H-score formula = (0×P0)+(1×P1)+(2×P2)+(3×P3), with a total score ranging from 0 to 300 [17].

Statistical analysis

SPSS Version 27 was used for statistical analysis, presented as mean±SD and median (IQR). One-way analysis of variance (ANOVA) was performed for data that was normally distributed, while the Kruskal-Wallis was used for non-normally distributed data. A significance level of 0.05 was applied for all statistical tests.

Results

Effect of iopromide on BUN and SCr levels

Histological examination of the kidneys using hematoxylin-eosin staining is presented in **Figure 2**. No significant differences were found in BUN levels among groups C, E24, E48, and E72 (**Figure 3A**). Similarly, there was no difference in SCr between the groups before and after contrast administration (**Figure 3B**). Hence, for the BUN and SCr parameters, there were no significant differences before and after contrast administration.

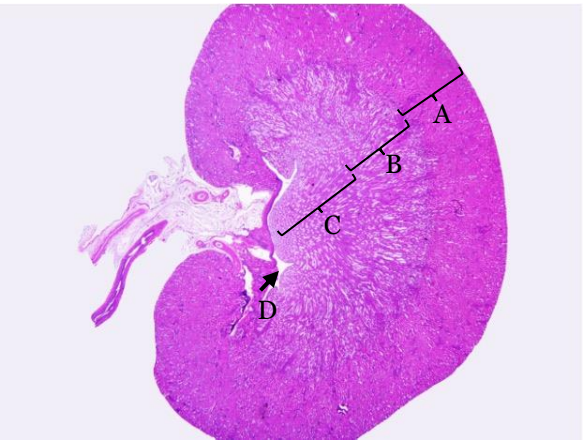

Figure 2. Kidney tissue stained with hematoxylin and eosin. The kidney rat with 2× magnification. (A) cortex; (B) medulla; (C) collecting ducts; and (D) renal pelvis.

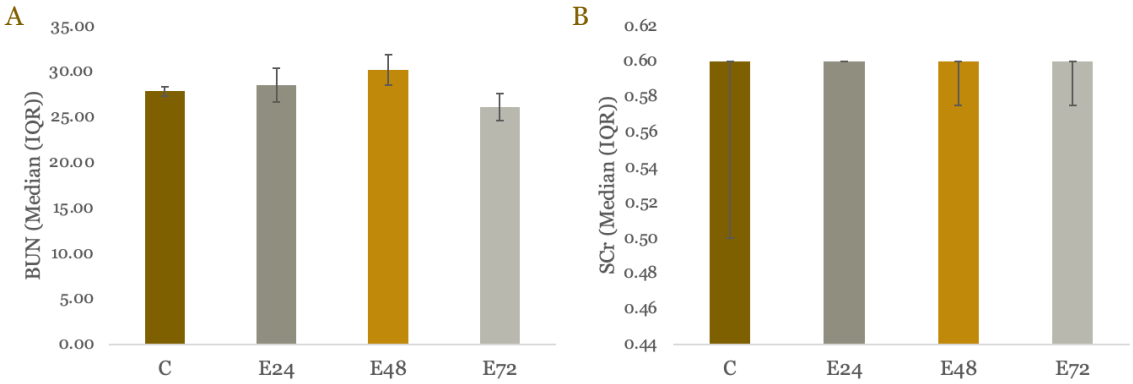

Figure 3. Bar charts of blood urea nitrogen (BUN) and serum creatinine (SCr) examination in four groups: (A) BUN examination: no significant difference between the four groups (*p*-value=0.181). (B) SCr examination: no significant difference between the four groups (*p*-value=0.677).

Iopromide effect on renal tubules: Loss of brush border, tubular necrosis, and proteinaceous casts

The histopathological examination score on the parameters of loss of brush border, tubular necrosis, and proteinaceous cast in the renal tubules is depicted in **Figure 4**. Renal tubule damage was found through the histopathological examination score shown by the parameter of

loss of brush border, indicated by an increased histopathological examination score when comparing group C with groups E24, E48, and E72 (**Figure 5A**). This study also found renal tubule damage on the tubular necrosis parameter that worsened after contrast administration, compared to group C with groups E24, E48, and E72 (**Figure 5B**).

However, the parameter of tubule damage related to the proteinaceous cast was different from the other parameters, such as loss of brush border and tubular necrosis. The histopathological examination of the renal tubules regarding the proteinaceous cast parameter showed damage in the first 24 hours. The proteinaceous cast examination then gradually improved after 72 hours, as shown by a significant decrease in the histopathological examination score between groups E72 and E24 (**Figure 5C**).

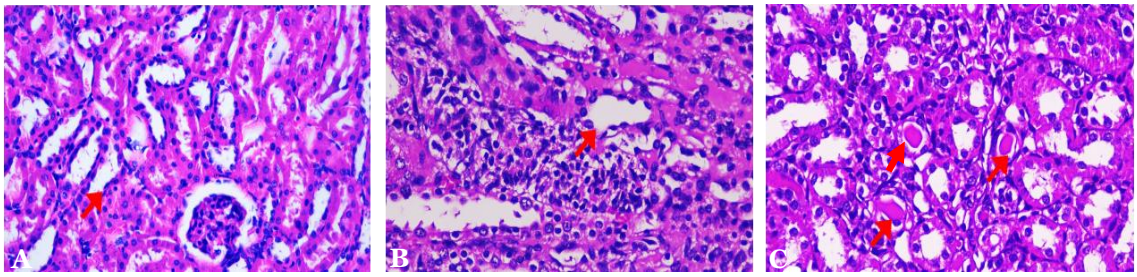

Figure 4. Sample of histopathological examination of kidney tissue stained with hematoxylin and eosin. Yellow arrows: (A) loss of brush border with flattening of epithelial cells, image scale at 500 µm; (B) tubular necrosis with loss of brush border, image scale at 50 µm; and (C) proteinaceous cast, image scale at 50 µm.

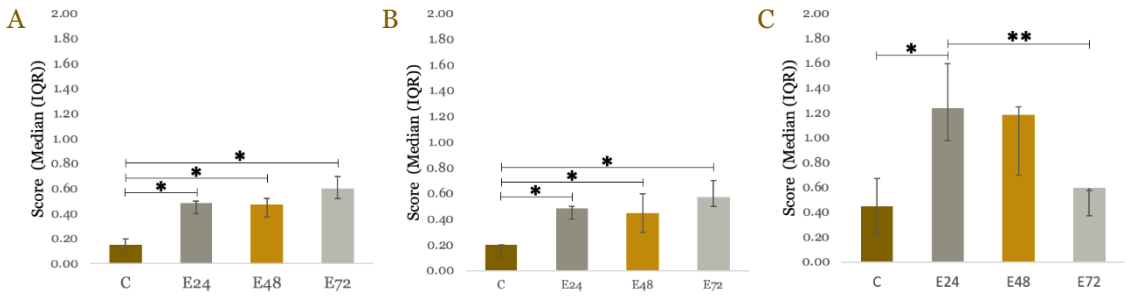

Figure 5. Bar charts of histopathological examination score in the renal tubules. (A) Histopathological examination score of loss of brush border appearance in the renal tubules: significant difference between four groups (p-value=0.001). *Significant difference between E24 and C (p-value=0.001), E48 and C (p-value=0.003), and E72 and C (p-value=0.001). (B) Histopathological examination score of tubular necrosis appearance in the renal tubules: significant difference between the four groups (p-value=0.001). *Significant difference between E24 and C (p-value=0.001), between E48 and C (p-value=0.002), and between E72 and C (p-value=0.001). (C) Histopathological examination score of proteinaceous cast appearance in the renal tubules: significant difference between the four groups (p-value=0.026). *Significant difference between E24 and C (p-value=0.023). **Significant difference between E72 and E24 (p-value=0.015).

Effect of iopromide on iNOS expression

Photographed images of the immunohistochemistry analysis on iNOS expression in the glomerulus, intrarenal vascular, and tubules are presented in **Figure 6**. The results were quantified using H-Score, where the values are presented in **Figure 7**. The data on iNOS in the glomerulus following iopromide contrast administration at 24 and 48 hours revealed a difference, as demonstrated by the differences between groups C and E24 and groups C and E48 (p=0.008 and p=0.005, respectively). The iNOS examination also found no difference in iNOS expression in the intrarenal vascular between the four groups (p=0.374), and there was no difference in iNOS expression in the tubules between the four groups (p=0.276).

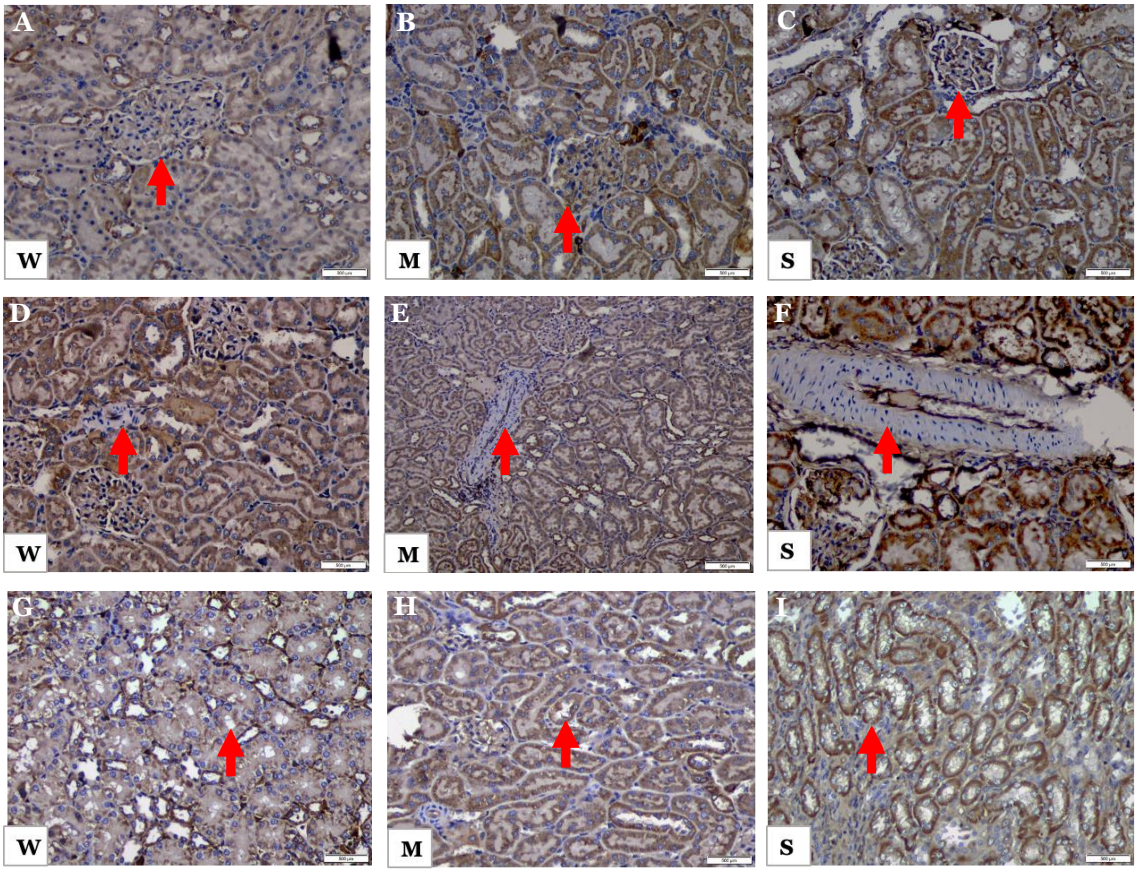

Figure 6. Sample of iNOS staining in the glomerulus (A-C), intrarenal vascular (D-F), and tubules (G-I). (W) Weak staining, image scale at 500 µm; (M) moderate staining, image scale at 500 µm; (S) strong staining, image scale at 500 µm. Red arrow: iNOS staining.

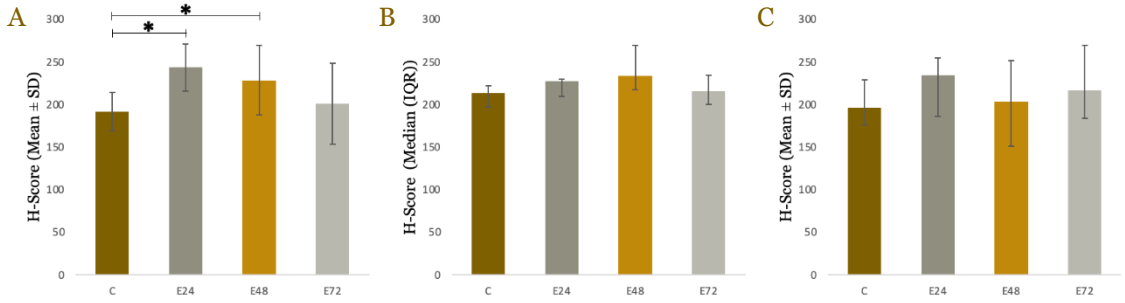

Figure 7. H-Score bar charts of iNOS expression in renal tissue. (A) iNOS expression in the glomerulus: significant difference between the four groups (p-value=0.027). *Significant difference between C and E24 (p-value=0.008) and C and E48 (p-value=0.005). (B) iNOS expression in the intrarenal vascular: no significant difference between the four groups (p-value=0.374). (C) iNOS expression in the tubules: no significant difference between the four groups (p-value=0.276).

Discussion

In this study, renal function was evaluated after performing a nephrectomy and administering iopromide. The findings indicated no statistically significant differences in BUN and SCr levels in the untreated group at 24, 48, and 72 hours. Previous research, including animal experiments with iopamidol, an iodine-based contrast agent, demonstrated similar results, showing no significant differences in serum creatinine levels compared to control groups at 24, 48, and 72 hours, similar to this study [18].

In contrast to the findings of this investigation, another study showed that the group receiving an injection of iopromide at a dosage of 7.5 mg/kg BB experienced contrast-induced

nephropathy (CIN) following exposure to contrast media, but none of the animals in the control group exhibited CIN. The treatment group exhibited a substantial rise in average serum creatinine levels compared to the control group at both the 24-hour and 48-hour time points. [19]. Within 48 hours, the average SCr level in the treatment group increased by 68.2% [19].

The data from this study and the data from another study showed no differences in serum creatinine excretion [18], possibly due to kidney tolerance to non-ionic contrast media with low osmolality given in this study, which has higher renal tolerance compared to ionic contrast medium with high osmolality [20]. Contrast media with low osmolality causes mild natriuresis and diuresis without triggering the activity of tubuloglomerular feedback. As a result, there is no constriction of the renal afferent arterioles and a decreased glomerular filtration rate [21]. Another potential scenario is that the malfunction of the tubular epithelium remains active, can still be reversed, and does not result in the blockage of the tubules [18].

The histopathological examinations in this study revealed significant differences in the criteria of loss of brush border and tubular necrosis. Furthermore, regarding the parameters of proteinaceous cast, there were significant differences between the C and E24 groups (p-value=0.023). However, no significant difference was detected between the C and E48 groups (p-value=0.122), nor between the C and E72 groups (p-value=0.442).

Prior research evaluated tubular damage in renal histopathology using a different methodology. In other investigations on histopathological morphology, acute tubular necrosis (ATN) is identified by the presence of edema and protein casts in the distal tubules and collecting ducts [18]. Another study conducted on rats to examine CIN found significant changes in kidney morphology after 3 days of administering iodine contrast at a dose of 4 g/kg body weight [16]. These changes included a significant increase in the loss of brush border, shedding of tubular cells, tubular dilation, and intratubular obstruction by granular casts detected in the rat kidneys compared to the control group [16].

The findings in this study showed that the parameters of tubular damage from proteinaceous cast improved 48 and 72 hours after contrast administration. The improvement in the proteinaceous cast parameters indicates an enhancement in the kidney tubule structure after two days of intervention. This finding differs from another study that reported damage after 72 hours or the third day [16,18]. These results are likely to be related to selected contrast material or to kidney function. Previous trials with mice undergoing uninephrectomy, furosemide injection, and LOCM of iohexol 10 mL/kg BB showed improvement in CIN within 3–7 days of treatment [22]. Another trial that tried to find models of mice with CIN showed kidney function through SCr and BUN parameters on the administration of iohexol and iodixanol contrast agents that improved at 72 hours [23].

The side effects of iodine-based contrast media also vary according to LOCM, including; ioversol, 1.8%; iohexol, 2.0%; iopamidol, 2.2%; iopromide, 3.5%; iomeprol, 3.9%; and the combination of these five, 2.7% among 8,931 patients undergoing CT scan with contrast, where the particular iodinated contrast media safety profiles differed significantly [24]. Further research is needed on the toxicity of each iodine contrast agent. Research conducted on rats with injections of different contrast agents, iohexol and iopamidol, at a dose of 1600 mg iodine/kg, showed no differences in SCr, BUN, and histopathological scores of tubular necrosis and proteinaceous casts [25].

The inconsistency between the functional states represented by BUN and SCr and the morphological parameters, such as the loss of brush border, tubular necrosis, and proteinaceous casts, raises questions in histopathological evaluation. This inconsistency is similar to previous studies, where SCr is considered a metabolic byproduct of muscle tissue and functions as an indirect measure of glomerular filtration rate efficiency [26]. SCr has poor predictive accuracy for kidney injury, especially in the early stages of acute kidney injury (AKI) [27]. In critical illness, SCr concentrations can fluctuate significantly due to factors such as dilutional volume status induced, the catabolic effects of critical illness, potential decreases during septic conditions, and increased tubular excretion as kidney function declines. Furthermore, after an injury event, the rise in SCr occurs slowly [27]. Iodinated contrast media are eliminated through glomerular filtration, filtered, and concentrated in the kidney tubules; this leads to tubular epithelial cells

being exposed to higher concentrations of iodinated contrast media, which causes damage to these cells [28].

The later parameters linked to iNOS were characterized by increased production of reactive oxygen species (ROS), inactivation of NOS, and prostacyclin synthases, which cause the renal blood vessels to contract and exacerbate the ischemia/hypoxia conditions in the kidney tissue. Excessive production of ROS also promotes oxidative stress and directly causes dysfunction of kidney and endothelial tubular epithelial cells, which together form a continuous cycle [2].

In this study, significant differences in the expression of iNOS were observed in the glomerulus between the sample groups. Subsequent post hoc analysis revealed significant differences between groups C and E24 (p-value=0.008), C and E48 (p-value=0.005), and between E24 and E72 (p=0.026). However, there was no significant difference between groups C and E72 (p-value=0.607). These findings indicate an increase in iNOS expression at 24 and 48 hours after the injection of iopromide in the renal glomerulus, followed by a decrease at 72 hours.

Several studies have demonstrated that iNOS is highly expressed in healthy kidney tissue, primarily in the tubules. In cases of chronic renal insufficiency, the expression of iNOS is significantly reduced [29]. Previous research has shown that iNOS expression is typically minimal or undetectable in healthy kidneys. However, certain kidney diseases have been linked to substantial levels of iNOS in both the glomeruli and interstitium of the kidney [29]. In another study utilizing an ischemic reperfusion model in a kidney, a 45-minute vascular occlusion followed by a 24-hour refusion resulted in elevated iNOS activity in the non-therapeutic group. However, the group undergoing reperfusion ischemic treatment exhibited a reduction in iNOS activity [30]. This study found that there were increased levels of glomerulus expression at 24 and 48 hours. These findings are consistent with other studies that have shown alterations in the morphology of renal cells, such as inflated glomerulus, loss of bowman space, and hypercellular mesangium, after one hour of administering iopromide [31].

The study revealed that there were no notable disparities in iNOS expression in the kidneys, except for the glomerulus within the initial 24 and 48 hours, when compared to the histopathological parameters of the tubules, including loss of brush border, tubular necrosis, and proteinaceous cast, which showed improvement after 48 hours.

According to a previous study, iNOS immunostaining showed its highest effectiveness between 6 to 18 hours after injection [32]. Subsequently, the effectiveness gradually decreased and reached a control level by day 7 [32]. Different studies observed iNOS on the third day following induction using iodine at 4 g/kgBB body weight [16]. However, the expression of iNOS was diminished when antioxidant agents were administered [16].

The lack of changes in iNOS expression in the tubules and vascular regions in this study may be attributed to the use of iopromide agents that contain low osmolality contrast media [33]. These agents do not stimulate tubuloglomerular feedback activity, resulting in the absence of vasoconstriction in the renal afferent arterioles and a decrease in the glomerulus filtration rate [18]. Consequently, this reduction in oxidative stress leads to a decrease in the synthesis of ROS and NO [2,34].

The limitations of this study are as follows: First, the use of animals that cannot fully replicate the process of CI-AKI in humans. Second, this study only uses one species or strain, which may not represent potential physiological differences. Third, further research is needed to investigate the effects of iopromide, specifically within the first 24 hours after contrast agent injection. Fourth, additional evaluation is required regarding glomerular damage, as indicated by iNOS expression in the glomerulus in this study.

Conclusion

This study found that renal function with parameters of BUN and SCr was not affected by the administration of iopromide. Iopromide increased renal tubular damage in the tubular brush border appearance and tubular necrosis appearance. However, tubular histopathology in proteinaceous casts improved after 48 hours. Iopromide also influenced the expression of iNOS in the glomerulus, with expression levels increased after 24 hours and decreased after 72 hours. In contrast, iNOS expression in the intrarenal vasculature and tubules remained unchanged.

These findings create possibilities for further research into the specific mechanisms and effects of iopromide on renal function, structure, and molecular expression.

Ethics approval

The Ethics Committee of the Faculty of Medicine at Universitas Airlangga, Surabaya, Indonesia, granted ethical approval for this study following the CIOMS 2016 guidelines. Registration number: 72/EC/KEPK/FKUA/2024.

Competing interests

The authors declare that there is no conflict of interest.

Acknowledgments

We express our gratitude to the Biochemistry Laboratories, Medical Faculty, Universitas Airlangga for their support during this study.

Funding

This research did not receive any external financial support.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Pranoto IW, Djojodimedjo T, Soebadi MA, *et al*. Effects of iopromide contrast agents on kidney iNOS expression and tubular histopathology alterations. Narra J 2024; 4 (3): e1227 - http://doi.org/10.52225/narra.v4i3.1227.

References

1. Lun Z, Liu L, Chen G, *et al*. The global incidence and mortality of contrast-associated acute kidney injury following coronary angiography: A meta-analysis of 1.2 million patients. J Nephrol 2021;34(5):1479-1489.
2. Li Y, Wang J. Contrast-induced acute kidney injury: a review of definition, pathogenesis, risk factors, prevention and treatment. BMC Nephrol 2024;25(1):140.
3. Guitterez N V, Diaz A, Timmis GC, *et al*. Determinants of serum creatinine trajectory in acute contrast nephropathy. J Interv Cardiol 2002;15(5):349-354.
4. Aubry P, Brillet G, Catella L, *et al*. Outcomes, risk factors and health burden of contrast-induced acute kidney injury: An observational study of one million hospitalizations with image-guided cardiovascular procedures. BMC Nephrol 2016;17(1):167.
5. Everson M, Sukcharoen K, Milner Q. Contrast-associated acute kidney injury. BJA Educ. 2020 Dec;20(12):417-423.
6. Lohani S, Rudnick MR. Contrast media—Different types of contrast media, their history, chemical properties, and relative nephrotoxicity. Interv Cardiol Clin 2020;9(3):279-292.
7. Al-Naimi M, Rasheed H, Hussien N, *et al*. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res 2019;10(3):95.
8. Stevens PE, Ahmed SB, Carrero JJ, *et al*. KDIGO 2024 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int 2024;105(4):S117-S314.
9. Fogo AB, Lusco MA, Najafian B, *et al*. AJKD atlas of renal pathology: Ischemic acute tubular injury. Am J Kidney Dis 2016;67(5):e25.
10. Dvanajscak Z, Cossey LN, Larsen CP. A practical approach to the pathology of renal intratubular casts. Semin Diagn Pathol 2020;37(3):127-134.
11. Songür HS, Kaya SA, Altınışık YC, *et al*. Alamandine treatment prevents LPS-induced acute renal and systemic dysfunction with multi-organ injury in rats via inhibiting iNOS expression. Eur J Pharmacol 2023;960:176160.
12. Zamora R, Vodovotz Y, Billiar TR. Inducible nitric oxide synthase and inflammatory diseases. Mol Med 2000;6(5):347-373.

13. Tasanarong A, Kongkham S, Itharat A. Antioxidant effect of Phyllanthus emblica extract prevents contrast-induced acute kidney injury. BMC Complement Altern Med 2014;14(1):138.
14. Institutional Animal Care and Use Program IOWA University. Vertebrate animal research anesthesia (Guidelines) 2023. Available from: https://animal.research.uiowa.edu/iacuc-guidelines-anesthesia. Accessed: 20 May 2024.
15. American Veterinary Medical Association. AVMA guidelines for the euthanasia of animals: 2020 edition. Available from: https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals. Accessed: 14 May 2024.
16. Aksu F, Aksu B, Unlu N, *et al*. Antioxidant and renoprotective effects of sphingosylphosphorylcholine on contrast-induced nephropathy in rats. Ren Fail 2016;38(7):1089-1098.
17. Nicholson RI, Bouzubar N, Walker KJ, *et al*. Hormone sensitivity in breast cancer: Influence of heterogeneity of oestrogen receptor expression and cell proliferation. Eur J Cancer Clin Oncol 1991;27(7):908-913.
18. Sukmaningtyas H, Trihadi DU. Pengaruh media kontras iopamidol dosis tinggi intravaskuler terhadap kadar kreatinin serum dan gambaran histopatologi tubulus ginjal pada tikus Sprague-Dawley: Upaya proteksi dengan l-arginin. M Med Indones 2008;43(3):137-147.
19. Mamoulakis C, Fragkiadoulaki I, Karkala P, *et al*. Contrast-induced nephropathy in an animal model: Evaluation of novel biomarkers in blood and tissue samples. Toxicol Rep 2019;6:395-400.
20. Wang YXJ, Jia YF, Chen KM, *et al*. Radiographic contrast media induced nephropathy: Experimental observations and the protective effect of calcium channel blockers. Br J Radiol 2001;74(888):1103-1108.
21. Heinrich MC, Kuhlmann MK, Grgic A, *et al*. Cytotoxic effects of ionic high-osmolar, nonionic monomeric, and nonionic iso-osmolar dimeric iodinated contrast media on renal tubular cells in vitro. Radiology 2005;235(3):843-849.
22. Wu J, Shen J, Wang W, *et al*. A novel contrast-induced acute kidney injury mouse model based on low-osmolar contrast medium. Ren Fail 2022;44(1):1346-1356.
23. Liu T, Luo W, Tan X, *et al*. A novel contrast-induced acute kidney injury model based on the 5/6-nephrectomy rat and nephrotoxicological evaluation of iohexol and iodixanol in vivo. Oxid Med Cell Longev 2014;2014:1-14.
24. Seong JM, Choi NK, Lee J, *et al*. Comparison of the safety of seven iodinated contrast media. J Korean Med Sci 2013;28(12):1703.
25. Asy'ari AH, Rahaju AS, Mustika A. Histopathology and renal function in wistar rats after intravascular injection of iodinated contrast media iohexol and iopamidol. Syntax Lit J Ilm Indones 2022;7(1):361.
26. Kiss N, Hamar P. Histopathological evaluation of contrast-induced acute kidney injury rodent models. Biomed Res Int 2016;2016:1-15.
27. de Geus HRH, Betjes MG, Bakker J. Biomarkers for the prediction of acute kidney injury: A narrative review on current status and future challenges. Clin Kidney J 2012;5(2):102-108.
28. Andreucci M, Faga T, Pisani A, *et al*. Acute kidney injury by radiographic contrast media: Pathogenesis and prevention. Biomed Res Int 2014;2014:1-21.
29. Fujihara CK, Mattar AL, Vieira JM, *et al*. Evidence for the existence of two distinct functions for the inducible NO synthase in the rat kidney. J Am Soc Nephrol 2002;13(9):2278-2287.
30. Yamasowa H, Shimizu S, Inoue T, *et al*. Endothelial nitric oxide contributes to the renal protective effects of ischemic preconditioning. J Pharmacol Exp Ther 2005;312(1):153-159.
31. Ikamaise VC, Ekanem TB, Obeten KE, Udo-Affah G. Ultravist studies on the histology patterns of the kidney of adult Wistar rats. Glob J Sci Front Res 2015;15(C2):1-7.
32. Choi JY, Nam SA, Jin DC, *et al*. Expression and cellular localization of inducible nitric oxide synthase in lipopolysaccharide-treated rat kidneys. J Histochem Cytochem 2012;60(4):301-315.
33. Zhang HY, Ji HX, Li W, *et al*. Correlation between bladder compliance and the content of detrusor collagen fibers. Chin Nurs Res 2016;3(2):83-85.
34. Güvenç M, Cellat M, Uyar A, *et al*. Nobiletin protects from renal ischemia-reperfusion injury in rats by suppressing inflammatory cytokines and regulating iNOS-eNOS expressions. Inflammation 2020;43(1):336-346.