

Rat renal ischemia-reperfusion model: From mechanistic insights to translational applications in acute kidney injury

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ABSTRACT

The renal ischemia-reperfusion (IR) injury model in rats is an indispensable experimental cornerstone for advancing our understanding of acute kidney injury (AKI). This model replicates the complex pathophysiology observed in critical human clinical scenarios such as renal transplantation, cardiovascular surgery, and septic shock, providing an unparalleled platform for mechanistic discovery and therapeutic screening. This review provides a critical analysis from a kidney physiology perspective, detailing the established surgical protocols—including bilateral clamping, unilateral clamping with and without contralateral nephrectomy, and advanced minimally invasive techniques. We dissect the phased pathophysiological cascade, from the initial ischemic metabolic crisis to the reperfusion-driven onslaught of oxidative stress, endothelial dysfunction, and inflammation, and correlate these events with functional and histological outcomes across different models. A rigorous appraisal of the model's strategic advantages, such as high physiological relevance and experimental control, is balanced against its inherent limitations, including species-specific differences and challenges in modeling chronicity. Finally, we present standardized frameworks for comprehensive diagnostic evaluation and robust preclinical therapeutic testing, cementing the rat IR model's role as a vital bridge between basic molecular research and clinical application in nephrology. Furthermore, this review highlights how recent refinements—such as modeling clinical comorbidities, applying multi-omics technologies, and incorporating novel biomarkers and imaging endpoints—are reshaping this foundational model's predictive utility for bridging the translational gap in AKI therapeutics.

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Introduction

Acute kidney injury (AKI) represents a formidable clinical syndrome characterized by a rapid decline in glomerular filtration rate, leading to the dysregulation of fluid, electrolyte, and acid-base homeostasis. Its incidence is rising globally, afflicting approximately 10-15% of all hospitalized patients and over 50% of those in intensive care units, thereby portending substantial morbidity, mortality, and healthcare burden (1-3). Renal ischemia-reperfusion (IR) damage is a significant and widespread cause of AKI, especially in perioperative and critical care settings (4). The pathophysiological paradox of IR injury is that although restoring blood flow is necessary for cellular salvage, reoxygenation itself paradoxically worsens tissue damage, triggering a cascade of events that prolongs the initial ischemic insult (4-6). Renal IR injury results from complex, multifactorial mechanisms, including tubular epithelial cell loss, vascular endothelial dysfunction, and a strong inflammatory response (7, 8).

Experimental models are essential given the ethical and practical limitations of researching the real-time, cell-specific dynamics of human AKI. The most important and physiologically relevant platform for analyzing these pathways remains the *in vivo* rat model of renal IR, induced by temporary clamping of the renal pedicles (9-11). This model accurately reproduces the main hemodynamic, tubular, and inflammatory characteristics of clinical ischemic AKI. This review offers a thorough “A-to-Z” analysis from a renal physiology perspective, describing the implementation, pathophysiological underpinnings, and use of this fundamental model in contemporary research. This review also presents a physiology-centered pathway for using the rat IRI model in translational AKI research by synthesizing basic and recent discoveries (12, 13).

Often called the “translational valley of death,” the shift from promising preclinical findings to successful clinical therapeutics for AKI remains a major obstacle despite decades of research (14, 15). This underscores the need for

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a physiologically sound model that also captures clinically relevant complexity. Recent developments are enabling new ways to close this gap, especially by modeling comorbid illnesses and applying multi-omics techniques within the rat IRI framework (16-18). Finally, this review incorporates these modern viewpoints, confirming the model's essential function and outlining a path toward more clinically predictive use. As a result, this review synthesizes current best practices for the rat IRI model, with a particular focus on variables that align with recent translational and precision-medicine frameworks. We stress the importance of moving beyond the typical healthy-animal paradigm by using clinically relevant functional and imaging goals, including comorbid diseases, and confirming results through spatial and single-cell omics. This approach allows the model to serve as a more faithful link between developing novel treatments for human AKI and discovering mechanisms.

Review methodology

This comprehensive narrative review broadly appraised the rat IRI model from a kidney physiology perspective. A structured, reproducible search strategy was employed to identify, evaluate, and synthesize the relevant scientific literature.

Search strategy and data sources

A thorough literature search was performed across several electronic databases, including PubMed/MEDLINE, Scopus, and Web of Science. The search was limited to articles published in English, with a primary focus on the time frame from January 2000 to June 2026, to capture modern advancements while including basic or principal older works cited within them (i.e., foundational studies that are repeatedly referenced in the more recent literature). The search utilized a combination of keywords and Medical Subject Headings (MeSH) terms related to the central topic. Key search terms included: ("renal" OR "kidney") AND ("ischemia-reperfusion injury" OR "IRI" OR "IR") AND ("rat" OR "rodent" OR "murine") AND ("model" OR "surgery" OR "pathophysiology") AND ("acute kidney injury" OR "AKI").

Studies were selected based on predefined inclusion and exclusion criteria

The initial search results were screened by title and abstract to identify potentially relevant articles. The full texts of these articles were then retrieved and assessed for eligibility against predefined inclusion and exclusion criteria.

Inclusion criteria

Studies were included in the current review if they examined IR injury and also satisfied at least one of the following additional criteria. First, the study had to provide detailed descriptions of the surgical methodologies employed. Second, the study was required to investigate pathophysiological mechanisms, including oxidative stress, inflammation, and cell death. Third, the study had to evaluate diagnostic biomarkers or assess therapeutic interventions. Fourth, the study had to be an original research article, an authoritative review, or an original experimental protocol.

Exclusion criteria

Studies were excluded from the current review if they

met any of the following criteria. First, studies that focused exclusively on other organ systems rather than the kidney were excluded. Second, studies that used only nephrotoxic AKI models without a direct ischemia-reperfusion injury component were not included. Third, the review excluded conference abstracts, editorials, and non-peer-reviewed publications. Fourth, studies that were not available in the English language were also omitted.

Data extraction and synthesis

Data from the selected articles were extracted into a standardized template, capturing information on the animal model (species, strain, sex), surgical technique (e.g., bilateral/unilateral clamping, ischemia duration), key pathophysiological findings, assessment methods (functional, histological, molecular), and major conclusions. Given the narrative review style, the data synthesis was thematic, focusing on comparing surgical models, elucidating the phased injury cascade, and critically evaluating the model's translational utility. The findings are presented in a structured format, supported by summary tables to enhance clarity and comparison.

Ethical considerations

This review is based on the analysis of previously published studies. All cited animal research was expected to adhere to the 3R principles (Replacement, Reduction, and Refinement) and to have been approved by the respective Institutional Animal Care and Use Committees (IACUC) of the original authors' institutions.

Surgical methodologies for inducing renal IRI

The choice of surgical model determines the type, extent, and translational context of the resulting injury. Each model has distinct benefits and limitations that must align with the research question. Table 1 presents a comparative summary of the four primary surgical methodologies.

Bilateral renal pedicle clamping

This model induces the most severe form of AKI, resulting in complete bilateral renal ischemia and rapid systemic azotemia, making it a model of profound, life-threatening renal failure (Table 1).

Detailed protocol

Following anesthesia (e.g., inhaled isoflurane or injectable ketamine/xylazine) and midline laparotomy, both renal pedicles are meticulously dissected and occluded simultaneously using non-traumatic microvascular clamps. The successful induction of ischemia is confirmed by visible kidney blanching. Core body temperature must be maintained at 37 °C using a homeothermic pad to prevent confounding hypothermic protection (11, 19).

Ischemia time and titration

The ischemia duration typically ranges from 30 to 60 min. The duration is directly and reproducibly proportional to injury severity and mortality. A 30-minute occlusion often produces moderate, reversible injury, whereas 45-60 min induces severe, often lethal AKI, characterized by massive tubular necrosis and a steep rise in serum creatinine (12, 20). This model is particularly useful for studying systemic inflammation and multi-organ crosstalk in severe AKI (21, 22).

Table 1. Surgical methodologies for inducing renal ischemia-reperfusion injury in rats

Model type	Surgical procedure	Ischemia duration	Primary applications	Key advantages	Key limitations	References
Bilateral Clamping	Occlusion of both renal pedicles simultaneously	30-60 min	Systemic consequences of severe AKI; multi-organ crosstalk	High reproducibility; clear functional endpoints	High mortality; intensive post-operative care	(11, 12, 19, 22)
Unilateral Clamping+Contralateral Nephrectomy	Unilateral nephrectomy followed by clamping of remaining kidney	25-45 min	AKI-to-CKD transition; therapeutic screening; long-term repair studies	Excellent survival; unambiguous functional assessment	Nephrectomy induces compensatory changes	(16, 25-29, 31, 32)
Unilateral Clamping Alone	Ischemia to one kidney; contralateral kidney intact	45-75 min	Molecular mechanisms; intrarenal signaling; paired analyses	Perfect internal control; no systemic uremia	Systemic SCr/BUN may remain near normal; limited sensitivity for the injured kidney	(10, 11, 16, 28, 33)
Selective Arterial Clamping	(i) Endovascular: femoral artery access with fluoroscopy. (ii) Laparotomy: isolation and selective clamping of renal artery alone	30-60 min (endo); 25-45 min (lap)	Molecular mechanisms; reduced surgical stress	Reduced trauma; preserves venous drainage; laparotomy variant needs no special equipment	Endo: expensive, technically demanding. Lap: meticulous dissection. Both less widely adopted	(24, 34, 35)

Reperfusion and post-operative care

The clamps are removed, and reperfusion is confirmed by the return of a bright red color to the kidneys. Post-operative care is intensive, requiring subcutaneous fluid resuscitation and close monitoring for signs of severe uremia (23).

Purpose and applications

This model is primarily used to study the systemic consequences of severe AKI (e.g., uremia, fluid overload, multi-organ crosstalk) and to test therapeutic interventions in a high-fidelity model of a global ischemic insult.

Advantages

The model offers high reproducibility of severe injury and clear functional endpoints (a dramatic rise in serum creatinine and blood urea nitrogen [BUN]).

Limitations

However, it is associated with high mortality with prolonged ischemia, requires intensive post-operative care, and does not mimic clinical scenarios where one kidney is spared (24, 25).

Unilateral clamping with contralateral nephrectomy

This is the most widely used and robust model, offering an optimal balance between injury severity and animal survivability (Table 1), and is considered the gold standard for many AKI studies (16, 26).

Detailed protocol

A unilateral nephrectomy is performed first, followed by clamping of the remaining kidney's pedicle. This approach forces the animal to rely solely on the injured kidney for survival, ensuring that functional parameters accurately reflect the injury in the ischemic organ (27, 28). This approach minimizes compensatory hyperfiltration confounders seen in unilateral models without nephrectomy (16, 29).

Ischemia time

Ischemia typically lasts 25 to 45 min. Slightly shorter times than the bilateral model are often used to control mortality while still achieving significant functional and histological injury (11, 16).

Purpose and applications

This model serves as the benchmark for studying the core pathophysiology of ischemic AKI, for high-throughput screening of renoprotective agents, and for investigating the long-term progression to fibrosis and chronic kidney disease (CKD) (11, 30, 31).

Advantages

The model provides excellent survival rates that permit long-term studies, and functional assessment is unambiguous because serum biomarkers depend solely on the injured kidney (11, 25).

Limitations

Contralateral nephrectomy induces compensatory hyperfiltration and other systemic changes that can independently confound injury and repair processes (32).

Unilateral renal pedicle clamping without contralateral nephrectomy

This model is best suited for deep mechanistic studies where a perfectly matched internal control is paramount, allowing isolation of intrarenal molecular events from systemic uremia (Table 1).

Detailed protocol

Only one kidney is subjected to ischemia, leaving the contralateral kidney intact and fully functional (28). The surgical procedure for inducing ischemia is identical to that used in other models (10, 11). Successful ischemia is confirmed by visual blanching of the kidney, and core body temperature is maintained at 37 °C with a homeothermic pad to prevent hypothermic protection (11, 19).

Ischemia time

The ischemia duration is often longer, ranging from 45 to 75 min, to ensure robust histological injury in the ipsilateral kidney, as the functional parameters serum creatinine (Scr) and BUN remain near normal due to compensation by the healthy contralateral kidney (16).

Reperfusion and post-operative care

The clamp is removed, and reperfusion is confirmed by

the return of a bright red color to the kidney. Post-operative care includes fluid resuscitation and standard monitoring. Because the contralateral kidney remains intact, the animal does not develop severe uremia, and post-operative care is less intensive than in bilateral or nephrectomy models (10, 11).

Purpose and applications

This model is particularly well suited for studying intrarenal molecular and histological changes (e.g., gene expression, phosphorylation signals, apoptosis), with the contralateral kidney serving as an internal comparator and thereby reducing inter-animal variability (33).

Advantages

It provides an ideal internal control for powerful paired statistical analyses and enables the study of repair mechanisms without the confounding effect of systemic uremia.

Limitations

Conventional systemic functional markers, such as serum creatinine and BUN, have limited sensitivity for assessing injury in the clamped kidney because the intact contralateral kidney compensates for the loss of function. Additionally, the healthy contralateral kidney may undergo compensatory changes that could influence systemic physiology (10, 11, 32).

Selective renal artery clamping

This technique offers a refined approach to inducing IR injury by selectively occluding the renal artery while minimizing surgical stress. Two principal approaches are available: an endovascular method and a laparotomy-based technique.

Detailed protocol

Endovascular approach

A microcatheter is advanced via the femoral artery under fluoroscopic guidance to selectively occlude the renal artery for 30–60 min, with successful occlusion and reperfusion confirmed by contrast angiography (34).

Laparotomy-based approach

The renal artery is carefully isolated from the vein and surrounding tissues via laparotomy, and a microvascular clamp is applied exclusively to the artery. This approach does not require fluoroscopy or specialized equipment (24, 35).

Ischemia time

30–60 min for endovascular; 25–45 min for laparotomy-based.

Reperfusion and post-operative care

Reperfusion is confirmed visually (laparotomy) or by angiography (endovascular). Both approaches require fluid resuscitation; the endovascular method reduces surgical stress.

Purpose and applications

Studying intrarenal molecular changes with minimal surgical trauma, preserving venous drainage, and mimicking clinical scenarios such as partial nephrectomy.

Advantages

Reduced surgical trauma, minimal inflammation, preserved venous drainage; the laparotomy variant is cost-effective and requires no specialized equipment.

Limitations

The endovascular approach is technically demanding and requires expensive equipment. The laparotomy-based method demands meticulous dissection. Both are less widely adopted than complete pedicle clamping (24, 35).

Pathophysiological mechanisms: A phased cascade of injury

The pathology of IRI unfolds in two interrelated phases: an ischemic phase that initiates damage through metabolic collapse, and a reperfusion phase that paradoxically amplifies the injury.

The ischemic phase: Metabolic crisis and initiation of injury

Upon renal artery occlusion, the resulting hypoxia triggers rapid, severe depletion of cellular adenosine triphosphate (ATP). This metabolic crisis drives all subsequent damage (4, 5).

Pathophysiology

The switch from aerobic to anaerobic metabolism leads to: (i) failure of ion pumps (Na^+/K^+ -ATPase and Ca^{2+} -ATPase), resulting in intracellular sodium and calcium overload, cellular swelling, activation of calcium-dependent proteases (calpains), and disruption of the actin cytoskeleton in proximal tubular cells, causing loss of polarity (7, 10, 36, 37); (ii) mitochondrial dysfunction, as the electron transport chain halts, leading to mitochondrial membrane depolarization and swelling, setting the stage for massive reactive oxygen species (ROS) generation upon reperfusion (7, 37); (iii) metabolic accumulation, where purine nucleotides degrade into hypoxanthine, which becomes a substrate for ROS-generating enzymes upon reperfusion (38, 39); and (iv) severity correlation, as the degree of ATP depletion is directly proportional to the duration of ischemia, with longer ischemic times leading to more profound bioenergetic failure and initiation of necrotic cell death (5, 40).

The reperfusion phase: Amplification of injury

The restoration of blood flow, while necessary for survival, facilitates the following destructive cascade that defines the severity of IR injury.

Oxidative and nitrosative stress

The sudden reintroduction of oxygen acts on the accumulated hypoxanthine via the enzyme xanthine oxidase, generating a burst of superoxide anions and hydrogen peroxide (5, 38, 39). Reactivating mitochondria also becomes a major source of ROS. This oxidative assault damages lipids, proteins, and DNA. Furthermore, ROS quench nitric oxide (NO), leading to the formation of peroxynitrite, a potent nitrosative agent that causes protein nitration and further cellular damage (5, 41).

Endothelial cell dysfunction and the “No-Reflow” phenomenon

Endothelial cells are early targets of injury. Oxidative stress

reduces the bioavailability of vasodilatory NO, promoting vasoconstriction. Adhesion molecules (P-selectin, E-selectin, and intercellular adhesion molecule-1 [ICAM-1]) are rapidly up-regulated on the endothelial surface (42-45). This leads to leukocyte rolling, adhesion, and transmigration. Critically, endothelial cell swelling and leukocyte plugging can cause “no-reflow,” a phenomenon where areas of the kidney, particularly the vulnerable outer medulla, fail to be reperfused despite patent arteries, perpetuating ischemia (46). The seminal work of Basile *et al.* has shown that endothelial dysfunction extends beyond the acute phase, with impaired proliferative capacity and a maladaptive mesenchymal transition contributing to chronic microvascular rarefaction (33, 47-49).

Innate immune activation and sterile inflammation

Dying tubular cells release damage-associated molecular patterns (DAMPs), such as high-mobility group box 1 (HMGB1) and ATP. These are recognized by pattern recognition receptors (e.g., Toll-like receptor 4 [TLR4]) on resident renal cells and infiltrating leukocytes, triggering a potent inflammatory response (4, 50). This leads to the production of pro-inflammatory cytokines (tumor necrosis factor- α [TNF- α], interleukin-1 beta [IL-1 β], and IL-6) and chemokines (monocyte chemoattractant protein-1 [MCP-1]), orchestrating the robust infiltration of neutrophils and macrophages, which directly damage tissue via proteases and additional ROS. Beyond the initial wave of neutrophil infiltration, the subsequent role of macrophages is now understood to be critically dynamic and heterogeneous. While the rat IRI model has been instrumental in defining the pro-inflammatory (M1) and pro-reparative (M2) paradigm, recent single-cell RNA sequencing studies have revealed a spectrum of macrophage states post-IRI, including a unique TREM2⁺ lipid-associated macrophage subset that emerges during the repair phase (51-54).

Tubular cell death and dysfunction

The combined metabolic, oxidative, and inflammatory insults trigger both necrosis (unregulated cell death) and apoptosis (regulated cell death). The S3 segment of the proximal tubule, located in the outer medulla—a region with naturally low oxygen tension—is exquisitely sensitive. Necrotic cells incite further inflammation while apoptotic cells and tubular casts contribute to tubular lumen obstruction and a subsequent reduction in glomerular filtration rate (GFR). Emerging forms of regulated cell death, including ferroptosis (iron-dependent lipid peroxidation) and necroptosis, have been characterized in this model, offering new therapeutic targets (4, 7, 55, 56).

The rat renal IRI model: Resolving critical knowledge gaps in human AKI

This model is a powerful, reductionist tool for probing individual elements of a highly complex pathophysiology, linking cellular biochemistry and whole-organ physiology. Accordingly, this model mainly contributes to:

Unraveling cell death pathways

The model allows for discriminating the contributions of apoptosis, necrosis, necroptosis, and ferroptosis to tubular injury, informing the development of targeted cytoprotective therapies (7, 55, 57, 58).

Decoding sterile inflammation

It has been instrumental in identifying Damage-associated molecular patterns (DAMPs), defining the sequential recruitment of neutrophils and macrophages, and establishing the pathogenic role of specific adhesion molecules and cytokines (50, 59-61).

Investigating microvascular dysfunction:

Techniques like intravital microscopy in the rat model have directly visualized the “no-reflow” phenomenon, leading to interventions aimed at protecting the endothelium (62). The model has been pivotal in identifying that the repair process itself can be maladaptive. Following IRI, a subset of tubular cells undergoes a partial epithelial-to-mesenchymal transition and enters a state of senescence, characterized by sustained cell cycle arrest in the G2/M phase and the secretion of a pro-inflammatory and pro-fibrotic secretome, the senescence-associated secretory phenotype (SASP) (63, 64). The rat IRI model has been used to test whether targeted clearance of these senescent cells with senolytic drugs (e.g., dasatinib and quercetin) can ameliorate fibrosis and improve renal function, revealing a novel therapeutic pathway to prevent the AKI-to-CKD transition (65, 66).

Elucidating tubular repair and the AKI-to-CKD transition

The model, particularly the unilateral non-nephrectomy variant, is well suited for long-term studies to map pro-repair signaling pathways and the mechanisms of maladaptive repair that lead to fibrosis (30). Additionally, recent spatial transcriptomics in rat IRI models has mapped the precise topographic distribution of pro-fibrotic signaling, identifying the cortico-medullary junction as a critical nexus for initiating maladaptive repair and fibroblast activation (54, 67, 68).

A critical appraisal: Strategic advantages versus inherent limitations

A sophisticated understanding of the model's strengths and weaknesses is paramount for experimental design and data interpretation. For example, the predominance of responsive M2 macrophages in rodents compared with humans may overstate intrinsic repair capacity, potentially explaining why many anti-inflammatory strategies successful in rats have failed in clinical trials. This difference highlights a basic problem: the rat model is good at showing how injuries happen, but its healing process may not reflect the long-term, out-of-control inflammation often seen in human patients with other health conditions. Therefore, data derived from the standard model must be interpreted with caution and ideally validated in comorbid or humanized systems before clinical translation.

Strategic advantages

The rat renal IRI model offers several strategic advantages that make it a valuable tool for AKI research. These include:

High physiological relevance

The rat kidney and the IR injury cascade closely mirror key aspects of human pathophysiology, providing strong translational face validity (4, 69, 70).

Precise experimental control

Key variables such as ischemic time, temperature, and

hydration can be standardized, allowing for titratable injury severity and robust cause-effect conclusions (27, 71).

Comprehensive tissue sampling

The kidney size permits extensive longitudinal and multi-omics analyses (histology, Western blot, RNA-seq) from a single animal, providing a holistic view of injury and repair processes (11, 72, 73).

Cost-effectiveness and established toolkit

The model is more feasible for large-scale studies compared to large animal models, with a vast repository of established pharmacological and genetic protocols.

Inherent limitations—anatomical and structural differences

While both rat and human kidneys have nephron heterogeneity, the distribution differs: rats have a higher proportion of short-looped nephrons, whereas human superficial nephrons have longer loops. These structural variations, along with differences in immune response, can limit direct extrapolation to humans (74).

Inadequate recapitulation of age-related and metabolic comorbidities

Standard IRI models typically use young, healthy animals and therefore fail to capture the diabetic (Table 2), aged, or hypertensive microenvironment where clinical AKI often occurs (10, 75, 76). To clarify the rationale for selecting the rat IRI model despite this limitation, Table 2 compares major preclinical AKI models by their strengths, limitations, and optimal use cases. For example, diabetes worsens IRI through two main mechanisms: impaired mitochondrial function and the accumulation of advanced glycation endproducts (AGEs), which trigger inflammation via their receptor (RAGE – receptor for advanced glycation endproducts) (77–79). Recognizing this limitation, the field is increasingly moving toward comorbid rat models to improve translational predictive value. Recent studies have successfully employed Zucker diabetic fatty (ZDF) rats, spontaneously hypertensive rats (SHR), and aged rodents to model IRI in the context of metabolic syndrome, hypertension, and aging, respectively (80–83). For instance, IRI in diabetic ZDF rats results in a markedly exaggerated inflammatory response, impaired mitochondrial biogenesis, and a more rapid progression to fibrosis compared to healthy controls, more accurately reflecting the human clinical scenario (79, 84, 85). These refined models are essential for testing the efficacy of therapeutic interventions, such as sodium-glucose cotransporter 2 (SGLT2) inhibitors

or senolytics, in a more translationally relevant context (86–89).

High intrinsic regenerative capacity

The rat kidney's robust self-repair can make modeling the progressive AKI-to-CKD transition challenging without using extreme injury protocols (30, 64, 90).

Technical variability and ethical considerations

Outcomes depend heavily on the surgeon's skill and experience, which can introduce variability between experiments. Additionally, the use of this model must strictly follow the 3R principles – Replacement, Reduction, and Refinement – to ensure ethical compliance and minimize animal suffering (91).

Standardized diagnostic and therapeutic frameworks

The model's validity depends on rigorous, multimodal assessment and a robust pre-clinical design for therapeutic testing.

Comprehensive diagnostic evaluation

A comprehensive diagnostic evaluation is essential for accurately characterizing the extent and nature of renal injury following IRI. This evaluation encompasses multiple assessment levels, as summarized in Table 3.

Intra-operative monitoring

During surgery, visual inspection of the kidney – which changes from pink to dark purple when blood flow is cut off and returns to pink when flow is restored – provides a quick check that the main blood vessel is successfully blocked and reopened. Laser Doppler flowmetry goes a step further by precisely measuring blood flow at the tissue level. This is important not only to confirm successful ischemia and reperfusion, but also to detect the “no-reflow” phenomenon, where tiny blood vessels fail to reopen even after the main artery is unblocked. Measuring this is crucial because untreated no-reflow can worsen injury and affect study results (27, 62, 92).

Functional and biochemical assessment: This includes:

Cornerstone biomarkers

Serum creatinine (S_{Cr}) and BUN are cornerstone biomarkers, peaking at 24–48 hr (12).

Direct GFR measurement

Creatinine clearance—derived from serum creatinine levels and urine collected over a defined period in metabolic cages – offers a more direct and quantitative assessment

Table 2. Comparative overview of animal models of acute kidney injury (AKI)

Model	Key advantage	Key limitation	Ideal use case	References
Rat IRI	Optimal balance of physiological relevance, cost, and tissue yield	Species differences; high innate regenerative capacity	Mechanistic studies; preclinical drug screening; transplantation immunology	(4, 11, 16, 69, 70)
Mouse IRI	Superior genetic toolbox (knockouts, transgenics)	Limited tissue size; more hemodynamically fragile	Unambiguous determination of a specific gene's role	(27, 71, 74)
Large animal IRI (e.g., Pig)	Close anatomical/physiological similarity to humans	Very high cost; ethical complexity; limited reagents	Translational validation; surgical technique development	(24, 35)

Table 3. Multi-modal diagnostic assessment of renal ischemia/reperfusion injury (IRI)

Category/Type	Assessment level	Specific technique	Key markers/parameters	Timing	References
Functional	Serum analysis	Creatinine, BUN	GFR surrogate	Peak 24–48 h	(12)
Clearance methods	Clearance methods	Creatinine clearance, Inulin clearance	Direct GFR measurement	Any time point	(92–94)
Novel biomarkers	Urine/Plasma analysis	Urinary NGAL, KIM-1, TIMP-2, IGFBP7, suPAR, mitochondrial DNA	Early detection; pathway-specific injury	Hours before SCr rise	(95, 99, 102, 103)
Histological	Light microscopy	PAS / H&E staining	Tubular necrosis, cast formation, brush border loss (Jablonski score)	24 hr – 4 weeks	(107, 110)
Immunohistochemistry	Tissue staining	KIM-1, Vimentin, CD68, MPO, Cleaved caspase-3, Ki-67	Tubular injury, inflammation, cell death, proliferation	24 hr – 4 weeks	(12, 59, 90, 111–114)
Fibrosis staining	Tissue staining	Picrosirius Red / Masson's Trichrome, α -SMA	Interstitial fibrosis, myofibroblast activation	Week 2 – 8	(12, 115, 116)
Molecular	Gene expression	qRT-PCR	Inflammatory cytokines, stress markers	24 hr – 4 weeks	(4, 53)
Protein analysis	Protein analysis	Western blot, ELISA	Cytokine levels, apoptotic proteins	24 hr – 4 weeks	(4, 53)
Cellular	Flow cytometry	Single-cell suspension staining	Immune cell populations: neutrophils (Ly6G ⁺), macrophages (F4/80 ⁺ , CD86 ⁺ , CD206 ⁺), T lymphocytes (CD3 ⁺)	24 hr – 7 days	(59, 117–119)

of glomerular filtration rate (GFR) compared to serum creatinine alone. For precise functional assessment, especially in unilateral models without nephrectomy or for subtle changes, inulin clearance remains the gold standard for measuring GFR (92–94).

Novel functional and tubular biomarkers

The measurement of urinary neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) provides an early, sensitive indicator of tubular damage, often rising hours before serum creatinine (S_{Cr}) levels become elevated (95–97). Other promising biomarkers include tissue inhibitor of metalloproteinase-2 (TIMP-2) • insulin-like growth factor-binding protein-7 ([TIMP-2]•[IGFBP7]) for cell cycle arrest, and liver-type fatty acid-binding protein (L-FABP) for oxidative stress in proximal tubules (3, 98, 99). The diagnostic landscape is evolving further with the discovery of biomarkers that reflect specific pathological processes. For example, urinary or plasma soluble urokinase plasminogen activator receptor (suPAR) has emerged as a biomarker linked to inflammation and immune activation, predicting both AKI incidence and poor recovery (100–102). Additionally, biomarkers of mitochondrial dysfunction, such as cell-free mitochondrial DNA, are being explored as early indicators of severe tubular injury (102, 103).

Advanced imaging

The use of functional magnetic resonance imaging (MRI) techniques, such as blood oxygen level-dependent (BOLD) MRI, in rat models provides a non-invasive method to quantify regional renal hypoxia, a key driver of ongoing injury and fibrosis (104–106).

Systemic and local oxidative stress

The systemic redox state can be assessed by measuring levels of malondialdehyde (MDA), a product of lipid peroxidation, and the activity of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) in both plasma and renal tissue homogenates (12, 107–109).

Histological and immunohistochemical (IHC) evidence

Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining are definitive for grading structural damage (e.g., tubular necrosis, cast formation, brush border loss) using a standardized score (e.g., Jablonski score) (110).

Targeted immunohistochemical staining provides critical mechanistic insights, including:

Tubular injury and dedifferentiation

Staining for KIM-1 (kidney injury molecule-1, also known as HAVCR1 [hepatitis A virus cellular receptor 1]) is a highly sensitive and specific marker for injured proximal tubules. Vimentin staining highlights dedifferentiated tubular cells (12).

Inflammatory infiltrate

Staining for CD68 to identify infiltrating macrophages and myeloperoxidase (MPO) for neutrophils allows for the quantification and localization of the innate immune response (59, 111–113).

Cell death and proliferation

Staining for cleaved caspase-3 identifies apoptotic cells, while markers like Ki-67 label proliferating nuclei (59, 90, 114).

Fibrosis (for long-term studies)

Staining for alpha-smooth muscle actin (α -SMA), which marks activated myofibroblasts, along with collagen I/III detection using Picrosirius Red or Masson's Trichrome staining, is essential for quantifying interstitial fibrosis (12, 115, 116).

Molecular and biomarker analyses and flow cytometric evidence

Gene expression (quantitative real-time polymerase chain reaction [qRT-PCR]) and protein analysis (Western blot, enzyme-linked immunosorbent assay [ELISA]) of inflammatory cytokines, stress markers, and apoptotic proteins are essential. Flow cytometric analysis of digested renal tissue provides an unparalleled quantitative profile of

the inflammatory landscape (4, 53). Single-cell suspensions can be stained with antibody panels to precisely enumerate and characterize immune cell populations, including neutrophils (Ly6G⁺), macrophages (F4/80⁺, CD86⁺, CD206⁺)(Table 3), and T lymphocytes (CD3⁺)(59, 117-119).

Therapeutic intervention studies

The model is a premier platform for evaluating renoprotective strategies. A summary of therapeutic study design and emerging interventions is presented in Table 4.

Intervention timing

Treatments can be given before (preconditioning), during (perconditioning), or after (postconditioning) ischemia. Postconditioning, administered at reperfusion, is the most clinically relevant because it matches the typical patient scenario (120-123).

Robust study design

Proper controls—including shamoperated and vehicle-treated IRI groups—are essential. Studies should also test multiple doses and assess outcomes at several time points to capture the full injury and repair process.

Primary and secondary endpoints

Endpoints should cover functional (e.g., reduced S_{Cr}), histological (e.g., improved injury score, less fibrosis), and molecular (e.g., reduced inflammation and apoptosis) measures to provide a complete picture of treatment effects.

For long-term AKI-to-CKD studies, endpoints include fibrosis markers (e.g., transforming growth factor-beta [TGF- β], collagen deposition).

Recent therapeutic strategies tested in the rat IRI model have moved beyond broad anti-inflammatory or antioxidant approaches towards targeted molecular interventions (124-126).

The promising therapeutic interventions include:

Senolytics

Clearing senescent cells with drugs such as dasatinib

and quercetin, or the B-cell lymphoma 2 (BCL-2) inhibitor ABT-263, has shown efficacy in mitigating fibrosis in long-term IRI studies(65, 66, 127-129).

Mitochondrial therapeutics

Compounds that enhance mitochondrial biogenesis (e.g., SS-31 peptides) or mitophagy have demonstrated significant renoprotection by improving cellular bioenergetics and reducing ROS production(36, 130-132).

Epigenetic modulators

Inhibitors of histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) are being explored to reverse the maladaptive epigenetic reprogramming that underlies the AKI-to-CKD transition(133-135).

Gasotransmitters

The therapeutic application of hydrogen sulfide (H₂S) donors has shown potent anti-inflammatory and anti-fibrotic effects in recent rat IRI studies (Table 4), highlighting a novel cytoprotective pathway(136-138).

Anti-inflammatory agents

Targeted anti-inflammatory strategies, including anti-ICAM-1 antibodies and TLR4 antagonists, continue to be explored to reduce sterile inflammation(42, 50, 139).

Future directions and emerging paradigms

To fully realize the translational potential of the rat IRI model, several forward-looking strategies are emerging:

Humanized and comorbid models

Systematic use of diabetic, aged, and hypertensive rat strains to mimic the clinical AKI microenvironment (80, 81, 143-145).

Multi-omic integration

Combining spatial transcriptomics (67), proteomics, and metabolomics to map injury and repair with cellular and topographical resolution (54, 67, 146).

Table 4. Therapeutic study design framework, experimental phases, and emerging interventions in the rat renal ischemia-reperfusion model

Study phase	Time point	Key actions	Primary endpoints	Therapeutic class	Examples	Mechanism	References
Pre-ischemic	Day -1 to 0	Baseline sample collection; pre-treatment (if preconditioning study)	Baseline values	—	—	—	(120-122)
Intra-operative	Day 0	Clamp placement; drug administration at reperfusion	Confirmation of ischemia/reperfusion	—	—	—	(120-122)
Acute phase	24 h	Blood collection; optional sacrifice subgroup	Peak Scr/BUN; acute histology; inflammation	—	—	—	(12)
Early repair	Day 3-7	Blood collection; sacrifice subgroup	Functional recovery trend; proliferation	—	—	—	(30, 90)
Chronic phase	Week 4-8	Terminal blood and tissue collection	Long-term function; fibrosis (CKD)	—	—	—	(30, 31, 63, 64)
Senolytics	—	—	—	Dasatinib+Quercetin; ABT-263	Clear senescent cells (SASP reduction)	Prevent AKI-to-CKD transition	(65, 66, 127-129)
Mitochondrial therapeutics	—	—	—	SS-31 peptides; mitophagy enhancers	Improve bioenergetics; reduce ROS	Cytoprotection during reperfusion	(37, 130, 131, 140, 141)
Epigenetic modulators	—	—	—	HDAC inhibitors; DNMT inhibitors	Reverse maladaptive epigenetic reprogramming	Mitigate fibrosis progression	(133-135, 142)

Real-time monitoring

Implantable biosensors and functional MRI (e.g., BOLD, diffusion tensor imaging) for longitudinal, non-invasive assessment of renal oxygenation and microstructure (104, 105, 147, 148).

AI-driven phenotyping

Machine learning for automated histopathology scoring and biomarker pattern recognition to reduce subjectivity and enhance predictive modeling (149-151).

Complementary in vitro models

Using rat IRI findings to validate organ-on-chip and microphysiological systems that allow for high-throughput mechanistic screening (152).

These approaches will not only refine the model's predictive validity but also accelerate the identification of clinically applicable therapeutic targets.

Conclusion

The rat IRI model remains an indispensable, yet imperfect, instrument in the nephrologist's research toolkit. Its unparalleled strength lies in its ability to faithfully recapitulate the integrated physiological and molecular cascades of human ischemic AKI within a controlled, accessible, and cost-effective system. A deep understanding of each surgical approach, coupled with rigorous multimodal assessment of injury, allows researchers to leverage this model to unravel fundamental mechanisms and evaluate promising therapeutics. However, the discerning investigator must always interpret findings through the critical lens of the model's limitations, particularly species-specific physiology and the absence of clinical comorbidities, to ensure the translational relevance and ultimate success of their work in the ongoing battle against acute kidney injury. To bridge the translational valley, the future of the rat IRI model lies not in its standard form, but in its refined application in comorbid contexts, coupled with multi-omics validation and clinically relevant endpoints such as functional MRI and novel biomarkers.

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Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Ethical Approval

This manuscript is a narrative review based on publicly available literature and does not involve any new human or animal research conducted by the authors. Therefore, ethical approval and informed consent were not required.

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GR M, H F, and T S assisted with resourcing, drafting the initial version, and conducting formal analysis. Z A contributed to resourcing and visualization efforts. GR M, H F, T S, and Z A participated in resourcing, drafting, and editing. CA S was responsible for conceptualization, validation, supervision, manuscript review and editing, formal analysis, and project management. This paper is submitted with the agreement of all its co-authors, and the author list has been approved by all authors.

Conflicts of Interest

The authors declare no competing interests that may have influenced this manuscript.

Declaration

The authors declare that Artificial Intelligence (AI) tools and large language models (LLMs) were used solely for grammatical correction, sentence rephrasing, and language refinement during manuscript preparation. The authors generated and validated all scientific content, concepts, data analysis, and interpretations with no reliance on AI for content creation.

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Corrected Proof