Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 23s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

The Role of N-Acetylcysteine (NAC) in Renal Ischemia-Reperfusion Injury

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Cite this paper as: Ryan Prasdinar Pratama Putra, Yan Efrata Sembiring, (2025) The Role of N-Acetylcysteine (NAC) in Renal Ischemia-Reperfusion Injury. *Journal of Neonatal Surgery*, 14 (23s), 832-841.

ABSTRACT

Background: Ischemia-reperfusion (IR) injury is a pathological process that exacerbates tissue damage upon the restoration of blood flow after ischemia. In the kidneys, IR injury can lead to acute tubular necrosis, endothelial dysfunction, and progressive renal impairment. N-acetylcysteine (NAC), a thiol-containing compound and precursor to glutathione, has shown promise as a nephroprotective agent due to its antioxidant and anti-inflammatory properties.

Methods: This review synthesizes findings from experimental studies and clinical trials investigating the effects of NAC in renal IR injury. A literature search was conducted across databases such as PubMed and ScienceDirect, focusing on studies that evaluated renal function markers, oxidative stress parameters, histopathological changes, and clinical outcomes following NAC administration.

Results: Animal studies demonstrate that NAC improves renal perfusion, reduces oxidative stress (e.g., lower MDA, MPO), and enhances antioxidant defenses (e.g., GSH, SOD, CAT). Histopathological evaluation revealed less tubular necrosis and interstitial damage in NAC-treated groups. Clinical trials in renal transplant recipients indicate a potential benefit in reducing early graft dysfunction and biomarkers of tubular injury, although findings remain inconsistent. Notably, prolonged use of NAC in chronic ischemic settings may alter redox homeostasis unfavorably, potentially worsening outcomes.

Conclusion: NAC exhibits beneficial effects in mitigating renal damage from acute ischemia-reperfusion injury via its antioxidant and anti-inflammatory actions. However, its long-term use in chronic or prolonged ischemia remains controversial and requires further investigation to clarify its therapeutic window and clinical applicability.

Keywords: *N-acetylcysteine, renal ischemia-reperfusion injury, oxidative stress, antioxidant therapy, acute kidney injury, nephroprotection*

1. INTRODUCTION

Ischemia is a condition characterized by a decreased blood supply to tissues, leading to a deficiency of oxygen and other essential nutrients required for cellular metabolism and survival. Restoring blood flow is an immediate intervention aimed at rescuing tissues from ischemic injury. However, the sudden restoration of blood supply following prolonged ischemia can lead to more severe complications, commonly referred to as ischemia-reperfusion (IR) injury (1,2). The initial ischemic insult results in tissue hypoxia. During prolonged ischemia, metabolic by-products accumulate within the cells. Upon reperfusion, local inflammation and the production of reactive oxygen species (ROS) are significantly increased, thereby exacerbating tissue injury. The resulting cellular damage from IR injury ultimately leads to apoptosis, autophagy, necrosis, and necroptosis (2,3).

Ischemia-reperfusion (IR) injury can affect various tissues and organs, including the kidneys. IR injury may result in sustained inadequate oxygen delivery, accumulation of metabolites, and depletion of adenosine triphosphate (ATP). In the kidneys, this can lead to ischemic acute tubular necrosis (ATN) as well as epithelial and endothelial damage if adequate renal perfusion is not restored. Ultimately, these events can trigger cell death and inflammation, leading to renal dysfunction.

Tubular cell death via necrosis and apoptosis constitutes the primary mechanism associated with renal IR injury (4).

Medical science continues to explore novel therapeutic strategies to prevent IR injury. Although many therapeutic approaches have shown beneficial effects in controlled experimental models, most have either produced inconclusive results in clinical practice or have yet to progress to human clinical trials. The use of antioxidants represents one treatment option to prevent the worsening of IR-induced injury. N-acetylcysteine (NAC) has been demonstrated to possess antioxidant effects that can attenuate IR injury (5). NAC is a widely used drug for treating acetaminophen overdose and as a mucolytic agent. Its primary role is linked to its antioxidant and anti-inflammatory properties, which support the maintenance of cellular redox balance. For this reason, its therapeutic potential spans a range of diseases in which oxidative stress plays a central role in their etiology and progression (6,7).

Previous studies have demonstrated the benefits of NAC in mitigating IR injury in various organs, including the heart, skeletal muscle, kidneys, lungs, and liver. NAC can reduce oxidative stress and inflammation, and it helps prevent apoptosis or cell death resulting from IR injury (8–11). In renal IR injury, NAC has been shown to improve renal blood flow and creatinine clearance by reducing oxidative metabolites and enhancing antioxidant capacity (12). Additionally, NAC may prevent renal dysfunction by decreasing oxidative stress and inflammatory markers (13). This review will discuss the role of NAC in mitigating the effects of renal ischemia-reperfusion injury.

2. ISCHEMIA-REPERFUSION (IR) INJURY

Pathophysiology of IR Injury

The overall tissue damage caused by ischemia-reperfusion (IR) injury can be divided into two phases: ischemic injury and reperfusion injury. The ischemic phase initially leads to tissue hypoxia and nutrient deprivation. During prolonged ischemia, cellular metabolic by-products accumulate, resulting in metabolic acidosis. Upon the restoration of blood supply (reperfusion), local inflammation and the production of reactive oxygen species (ROS) significantly increase, causing secondary injury (Naito et al., 2020; Wu et al., 2018). The general pathophysiology of IR injury is summarized in Figure 1. In IR injury, time is a critical factor: the longer the delay in restoring oxygen flow and pressure, the greater the extent of tissue damage (14).

Ischemic Injury

1. Anaerobic Metabolism

The primary function of mitochondria in cells is to produce adenosine triphosphate (ATP) through oxidative phosphorylation. Ischemic conditions (reduced oxygen supply) induce a metabolic shift from aerobic to anaerobic metabolism within the mitochondria, leading to dysfunction of the electron transport chain (14). This reduces oxidative phosphorylation and subsequently impairs the synthesis of high-energy molecules such as ATP and phosphocreatine (Kumar et al., 2020; Naito et al., 2020). Anaerobic metabolism increases the production of lactic acid, thereby lowering intracellular pH and creating an acidic environment that is suboptimal for the function of many cellular enzymes (Wu et al., 2018).

2. Disruption of Ion Homeostasis

ATP depletion results in the failure of ATP-dependent ion exchange pumps, such as the sodium-potassium pump (Na^+/K^+-ATPase) and the calcium pump (Ca^{2+}-ATPase) located on the cell membrane (Naito et al., 2020; Wu et al., 2018). Dysfunction of the Na^+/K^+-ATPase pump causes intracellular sodium retention and extracellular potassium accumulation. Increased intracellular sodium levels reduce the activity of the sodium-hydrogen exchanger (Na^+/H^+ pump), leading to intracellular hydrogen ion accumulation. The resulting intracellular acidosis impairs enzymatic activity. Additionally, dysfunction of the Ca^{2+}-ATPase pump in the endoplasmic reticulum inhibits calcium reuptake into intracellular stores, leading to cytoplasmic calcium overload. This causes hyperosmolarity and subsequent cellular swelling, ultimately disrupting cellular homeostasis (Naito et al., 2020; Sánchez, 2019).

3. Hypoxanthine Accumulation

ATP depletion also contributes to the accumulation of hypoxanthine in ischemic tissues (Kumar et al., 2020). Hypoxanthine is a metabolic by-product of purine catabolism. Under acute energy demand, ATP is degraded into hypoxanthine. Hypoxanthine is normally oxidized to xanthine by xanthine dehydrogenase, and further converted to uric acid (Johnson et al., 2019; Kumar et al., 2020). However, during ischemia, xanthine dehydrogenase is converted into xanthine oxidoreductase/oxidase due to decreased ATP levels, leading to excess hypoxanthine accumulation in cells. This accumulation contributes to a surge in ROS production during the reperfusion phase (Kumar et al., 2020; Vishwakarma et al., 2017).

4. Activation of NADPH Oxidase

The NADPH oxidase (NOX/Duox) family contributes to ROS production during IR injury. NOX enzymes utilize oxygen as the final electron acceptor via NADPH, FAD, and heme groups. Overexpression and enhanced activity of NOX enzymes are

implicated in ROS generation in IR injury. NOX enzymes generate superoxide (O_2^-), which is further converted into hydrogen peroxide (H_2O_2). Superoxide leads to nitric oxide degradation, peroxynitrite formation, and tyrosine protein nitration. Hydrogen peroxide diffuses across membranes to oxidize cysteine residues, inactivate tyrosine phosphatases and the serine-threonine phosphatase calcineurin, react with peroxidases, and induce cellular toxicity (Wu et al., 2018).

5. Reduction in Antioxidant Levels

Anaerobic metabolism also leads to reduced production of endogenous antioxidants during the ischemic phase (Wu et al., 2018). Superoxide dismutase (SOD) is a key early antioxidant defense that neutralizes superoxide radicals and mitigates subsequent oxidative stress. Glutathione (GSH) is an endogenous tripeptide with antioxidant properties and metabolic functions. The potent antioxidant activity of GSH protects cells, particularly cell membranes, from free radical-induced damage. Experimental models of IR injury have demonstrated significant reductions in GSH levels, SOD activity, and other antioxidant defenses (Güler et al., 2022).

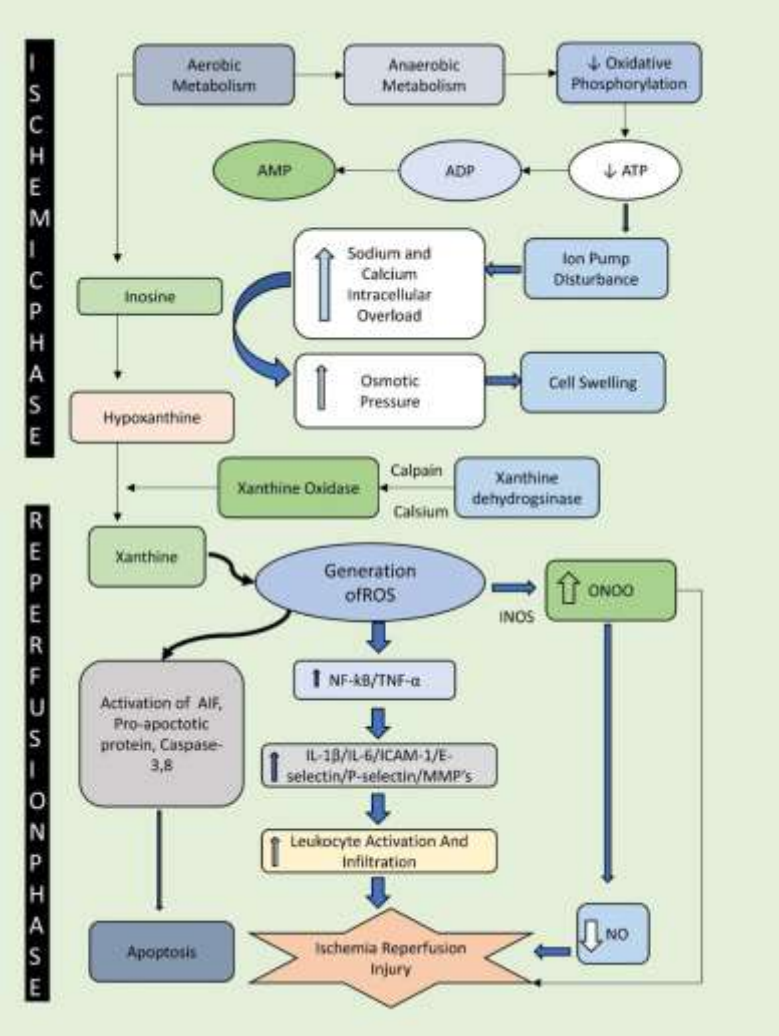

Figure 1. Pathological Mechanisms of Ischemia-Reperfusion Injury. Abbreviations: ATP: adenosine triphosphate; ADP: adenosine diphosphate; AMP: adenosine monophosphate; ROS: reactive oxygen species; AIF: apoptotic inducing factor; iNOS: inducible nitric oxide synthase; NF-κB: nuclear factor kappa-B; TNF-α: tumor necrosis factor-α; IL: interleukin; ICAM: intracellular adhesion molecule; VCAM: vascular cell adhesion molecule; MMP: matrix metalloproteinase; NO: nitric oxide (1).

Reperfusion Injury

1. Oxidative Stress

Oxidative stress can originate from enzymatic and non-enzymatic sources. Common enzymatic sources include the xanthine oxidase system, NADPH oxidase system, mitochondrial electron transport chain, and the uncoupled nitric oxide synthase (NOS) system. Non-enzymatic sources, such as hemoglobin and myoglobin—particularly in limb injuries—contribute to a

smaller extent (Wu et al., 2018). During the reperfusion phase, restoration of blood flow provides oxygen supply to ischemic tissues. This oxygen is utilized by xanthine oxidase to convert accumulated hypoxanthine from the ischemic phase into xanthine (Kumar et al., 2020; Wu et al., 2018). This conversion process generates large amounts of reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), thus exacerbating oxidative stress (Wu et al., 2018). Oxidative stress also results from decreased antioxidant defenses in ischemic tissue (Güler et al., 2022). This stress contributes to endothelial dysfunction, DNA damage, and localized inflammatory responses (Wu et al., 2018). ROS accumulation can further trigger lipid peroxidation, which damages cell membranes and causes cellular swelling (Naito et al., 2020; Vishwakarma et al., 2017).

2. Inflammation

Inflammation may be initiated by ROS and calcium accumulation during ischemia. Ischemia-reperfusion (IR) injury provokes a complex inflammatory response in the absence of pathogens—a process referred to as sterile inflammation (Güler et al., 2022). This type of inflammation is mediated through signaling events involving pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), recruitment of innate and adaptive immune cells, and activation of the complement system (Naito et al., 2020; Sánchez, 2019). PRRs are activated upon binding to danger-associated molecular patterns (DAMPs) such as peroxiredoxins, nucleotides, purines, and nucleic acid fragments. This interaction activates inflammatory transcription factors such as nuclear factor kappa-B (NF-κB), activator protein-1 (AP-1), and mitogen-activated protein kinases (MAPKs). The activation of these molecules leads to the release of inflammatory cytokines, chemokines, adhesion molecules, matrix metalloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS), and nitric oxide (NO), all of which contribute to further tissue damage (Güler et al., 2022; Naito et al., 2020). Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) are elevated in various studies on IR injury and play critical roles in acute inflammation (Güler et al., 2022).

3. Autophagy and Cell Death

Autophagy, a cellular process responsible for degrading damaged or unnecessary organelles and proteins, also contributes to the pathogenesis of IR injury. ROS accumulation leads to membrane and organelle degradation and single-strand DNA damage due to enzyme inactivation, along with activation of (ADP-ribose) polymerase, resulting in cell death through necrosis, apoptosis, autophagy, mitoptosis, and necroptosis (Naito et al., 2020; Vishwakarma et al., 2017). Moderate ischemia-reperfusion injury may induce autophagic responses and trigger cell survival mechanisms. However, in severe cases, irreversible damage results in apoptotic or necrotic cell death (Naito et al., 2020; Wu et al., 2018). Necrosis is characterized by cellular disintegration, organelle swelling, and mitochondrial dysfunction, which collectively elicit a strong local inflammatory response in the affected tissues (Wu et al., 2018).

Renal Injury due to Ischemia-Reperfusion (IR)

Ischemia-reperfusion (IR) injury can lead to sustained inadequate oxygen delivery, accumulation of metabolites, and depletion of ATP. In the kidneys, this condition may result in ischemic acute tubular necrosis (ATN), as well as epithelial and endothelial damage if adequate renal perfusion is not restored. Ultimately, this can lead to cell death and inflammation, causing renal dysfunction (Figure 2). Tubular cell death through necrosis and apoptosis is a key mechanism associated with renal IR injury (4).

Renal Epithelial Cells Injury

When blood flow to the kidney is reduced, intracellular ATP levels in epithelial cells decrease, which can result in cellular injury or death. Although any part of the nephron may be affected, proximal tubular epithelial cells (TECs) are particularly vulnerable due to their high metabolic activity and limited capacity for anaerobic energy production. Ischemic injury to proximal TECs is considered a primary cause of acute kidney injury (AKI). Damage to these cells causes loss of the apical brush border, exposing denuded areas of the tubular basement membrane, and can lead to proximal tubular dilation (4).

Renal Endothelial Dysfunction

Endothelial cells play a vital role in regulating vascular tone, blood flow, coagulation, inflammation, and permeability. IR injury can cause endothelial swelling and cytoskeletal degradation, which subsequently disrupts tight junctions between endothelial cells, increases vascular permeability, and results in fluid leakage into the interstitial space. This vascular leakage triggers increased intravascular viscosity, hemostasis, and vascular congestion—predominantly localized in the outer medulla (15,16). Post-ischemic renal arterioles often undergo vasoconstriction due to reduced production of vasodilators by injured endothelial cells and elevated levels of vasoactive cytokines. The endothelium releases vasoactive substances such as platelet-derived growth factor (PDGF) and endothelin-1 (ET-1). Additionally, the number of microvessels in the outer medulla is reduced following IR injury, potentially leading to chronic hypoxia, further tubular injury, and fibrosis (4,15).

Vasoconstriction may be exacerbated by decreased production of nitric oxide (NO) during reperfusion, owing to downregulation of endothelial nitric oxide synthase (eNOS), along with increased arteriolar sensitivity to vasoactive substances such as angiotensin II, thromboxane A2, and prostaglandin H2. Furthermore, ischemic injury may alter the

intrarenal distribution of renal blood flow (RBF) during reperfusion. In severe injuries, persistent reductions in RBF may occur, leading to the so-called "no-reflow" phenomenon—characterized by inadequate perfusion at the microcirculatory level despite reperfusion. The regenerative capacity of endothelial cells in peritubular capillaries is limited, and injury to this microcirculation may result in permanent damage to the peritubular capillaries (15,16).

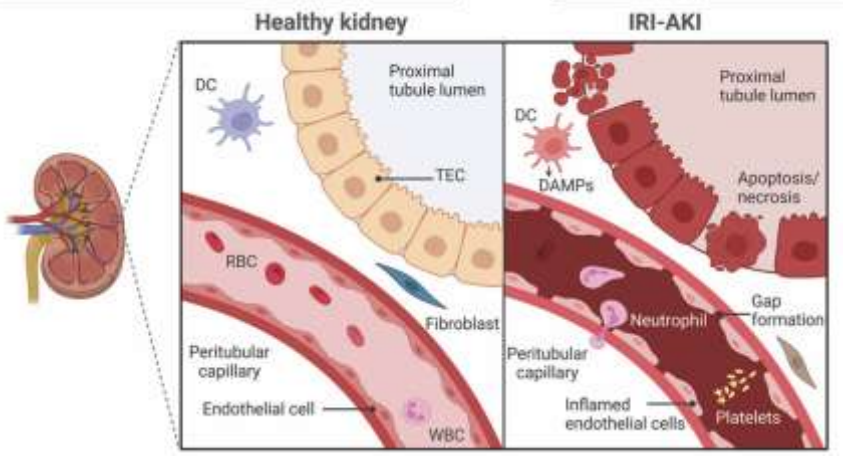

Figure 2. Illustration of a kidney affected by IR (ischemia-reperfusion) injury (cortical area).

IR injury can damage kidney cells and disrupt kidney function, leading to acute kidney injury (AKI). This may result in interstitial widening, microvascular obstruction, tubular dilation, and patchy injury patterns, ultimately causing cell death. TEC: tubular epithelial cell, DC: dendritic cells, RBC: red blood cell, WBC: white blood cell, DAMPs: damage-associated molecular patterns (4).

Renin-Angiotensin System (RAS) Activation

Among the various mediators involved in ischemia-reperfusion (IR)-induced kidney injury, the renin-angiotensin system (RAS) plays a critical role. IR injury is known to alter or modulate the balance of RAS components. In the kidneys, RAS components are distributed across the tubular structures, interstitial tissue, and intracellular compartments (17). ctivation of the RAS and elevated levels of angiotensin II are major risk factors in IR injury. Through stimulation of AT1 receptors (AT1R) in injured kidneys, angiotensin II promotes the expression of pro-inflammatory genes and triggers tubulointerstitial fibrosis. Angiotensin II is also known to contribute to renal injury through vasoconstriction of renal vessels, increased vascular sensitivity to sympathetic stimulation, oxidative stress enhancement, and the induction of apoptosis. Moreover, angiotensin-converting enzyme (ACE) inhibitors have shown protective effects against IR-induced kidney damage through their antioxidant, anti-apoptotic, and anti-inflammatory properties—reducing protein oxidation, lipid peroxidation, and acute tubular injury (17,18).

Preventive Management of Ischemia-Reperfusion Injury

Medical science has been striving to develop novel therapeutic strategies to prevent IR injury. Although many of these strategies have proven beneficial in controlled experimental models, most have shown inconclusive results in clinical practice or have yet to reach human clinical trials. Therefore, timely reperfusion remains the cornerstone of current clinical practice. Repeated exposure to short, non-lethal episodes of ischemia can provide protection to tissues against prolonged IR injury. This phenomenon is known as ischemic preconditioning (IPC). In myocardial I-R experimental models, IPC has been demonstrated to restore ventricular function, reduce apoptosis, and limit neutrophil accumulation in myocardial tissue (5).

Mild to moderate hypothermia therapy has also exhibited significant protective effects against IR injury without severe complications. Regional hypothermia at 10°C in a rabbit model of limb injury resulted in increased ATPase activity and reduced levels of potassium, lactate, and inflammatory cytokines. IPC, which involves a brief period of ischemia followed by short reperfusion before prolonged ischemia, has been reported to alleviate internal organ IR injury in both experimental and clinical studies (3)

As previously discussed, excessive oxidative stress is one of the primary mechanisms underlying IR injury. Several compounds—such as N-acetylcysteine, angiotensin-converting enzyme inhibitors, iron chelators, catalase, mannitol, superoxide dismutase, allopurinol, melatonin, vitamins C and E, and calcium channel blockers—have demonstrated antioxidant properties that may reduce IR-induced damage (3,5). Specific strategies to mitigate renal reperfusion injury include renal replacement therapy and metabolic management. Metabolic consequences of reperfusion can be addressed through hyperventilation, membrane stabilization using calcium and/or magnesium, ionic buffers such as sodium bicarbonate, and administration of intravenous glucose with insulin if necessary. Early hemodialysis remains the treatment

of choice in patients presenting with anuria accompanied by metabolic acidosis and hyperkalemia (19).

N-acetylcysteine (NAC)

N-acetylcysteine (NAC) is a widely used drug for the treatment of acetaminophen (paracetamol) overdose and as a mucolytic agent in respiratory tract diseases (20). NAC is a synthetic derivative of the endogenous amino acid L-cysteine and a precursor of glutathione (GSH). NAC not only modulates oxidative stress but also other pathophysiological processes involved in diseases, including mitochondrial dysfunction, apoptosis, inflammation, and indirect effects on neurotransmitters such as glutamate and dopamine (21). N-acetylcysteine is a safe, inexpensive, and well-tolerated antioxidant with a well-established mechanism of action. Due to its highly favorable risk/benefit ratio and low rate of side effects, it is widely used (22). However, despite being a well-known antioxidant and a long-standing generic drug with several established clinical applications, its potential uses remain inadequately investigated (20).

Pharmacology of NAC

NAC can be administered orally, intravenously, or via inhalation, and is generally safe and well-tolerated, even at high doses. Intravenous NAC is used for the treatment of paracetamol overdose via continuous infusion with gradually tapered doses. Dosage protocols vary between regions (21,23). For oral NAC administration, no standardized dosage has been recommended, resulting in a wide dosage range used in clinical trials. In Australia, the maximum over-the-counter dosage recommended is 1000 mg/day; however, previous studies have shown that oral NAC doses up to 3600 mg/day are still well-tolerated (23).

The chemical structure of NAC consists of a sulfhydryl functional group (–SH) and an acetyl group ($–COCH_3$) attached to an amino group (NH_2) (7). Orally administered NAC undergoes intestinal absorption and hepatic metabolism, mainly resulting in the release of cysteine for GSH synthesis. After oral administration, the maximum plasma concentration (Cmax) occurs approximately between 1 and 2 hours. The bioavailability of free NAC is very low (<10%), and only a small amount of intact molecules reach the plasma and tissues. NAC largely circulates in the blood bound to proteins (66–87%). Serum concentrations following intravenous administration at an initial dose of 150 mg/kg over 15 minutes reach approximately 500 mg/L. The volume of distribution ranges between 0.33 and 0.47 L/kg (6,21).

NAC is metabolized into N-acetylcystine and N,N-diacetylcystine dimers, which may be deacetylated to form cysteine. Following complete metabolism of NAC, cysteine, cystine, inorganic sulfate, and glutathione are the major metabolic products. Due to the bypass of intestinal and hepatic metabolism, intravenous administration enables rapid delivery of high-concentration NAC (6,21,23). After inhalation or intratracheal administration, most of the administered NAC participates in sulfhydryl-disulfide reactions, while the rest is absorbed by the pulmonary epithelium and deacetylated by the liver into cysteine for metabolism. The half-life of NAC in adults is about 5.6 hours. The average clearance is approximately 0.11/hour/kg. NAC is excreted 13–38% via urine and 3% via feces (6,7).

Side Effects of NAC

The side effects of NAC range from mild to severe and depend on the formulation and dose used. Intravenous and oral NAC are generally associated with minimal side effects. The toxicity of NAC overdose has not been established for patients with paracetamol overdose or for healthy individuals taking single or repeated doses of NAC (6,7). At oral doses of 1200 mg twice daily or lower, side effects are rare, although some mild to moderate gastrointestinal side effects may occur, including nausea, vomiting, diarrhea, bloating, epigastric pain, and constipation, or skin reactions such as transient rash or redness. At much higher doses used to treat acetaminophen overdose, NAC is often poorly tolerated, with side effects such as headache, tinnitus, urticaria, rash, chills, fever, and anaphylactoid (pseudo-anaphylaxis) reactions (22,24).

The Role of N-Acetylcysteine (NAC) in Ischemia-Reperfusion Injury

The primary role of N-acetylcysteine (NAC) is associated with its antioxidant and anti-inflammatory activities, which support the maintenance of cellular redox balance. For this reason, its therapeutic potential involves a range of diseases whose etiology and progression are related to oxidative stress (6). NAC exerts direct antioxidant effects on certain oxidative species. Its indirect antioxidant effect stems from its ability to act as a cysteine precursor, which is a key component in the synthesis of glutathione (GSH). NAC's capacity to disrupt disulfide bonds and restore thiol levels also contributes to redox state regulation (7,25). A summary of the protective mechanisms of NAC is illustrated in Figure 3.

Direct Antioxidant Effects

As a thiol compound, NAC can react with many radical and non-radical oxidants. However, for NAC to act as an antioxidant in biological matrices, its reaction rate with oxidants must be higher than that of endogenous antioxidants and significantly higher than that of substrate molecules. Therefore, based on reaction rate and concentration, NAC's antioxidant activity is not effective against certain oxidant species such as hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), and peroxynitrite ($ONOO^-$), due to their relatively low reactivity with NAC. Nevertheless, for other oxidative species—such as one- and two-electron oxidants including nitrogen dioxide (NO_2), hypochlorous acid (HOCl), and hydroxyl radicals (•OH)—NAC may exert a direct antioxidant effect (7,25).

Indirect Antioxidant Effects

Aktivitas

The indirect antioxidant activity refers to NAC's role as a precursor of GSH. GSH is the most abundant non-protein thiol in the body and one of the primary antioxidants responsible for maintaining cellular redox balance. It also functions as a cofactor or substrate for various antioxidant enzymes (6). Glutathione is a tripeptide (γ-L-glutamyl-L-cysteinylglycine, GSH) synthesized and maintained at high intracellular concentrations (in the millimolar range). The intermediate compound, γ-glutamylcysteine, is first synthesized from L-glutamate and cysteine via the enzyme γ-glutamylcysteine synthetase (also known as glutamate-cysteine ligase). L-glycine is then added to the C-terminal of γ-glutamylcysteine by glutathione synthetase (25). NAC enhances GSH synthesis by supplying cysteine (Cys), the rate-limiting substrate in cellular GSH biosynthesis. Deacetylation of NAC is catalyzed by several aminoacylase enzymes (I, II, and III), and NAC is hydrolyzed by cytosolic acylase I. The highest concentrations of NAC deacetylase activity have been found in the kidneys across all studied species (25).

In addition, gene regulation of endogenous antioxidant defense systems—including GSH synthesis—is also crucial. GSH production can be regulated by the transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2). NAC's protective effects against oxidative stress are also linked to the Nrf2 pathway, which promotes GSH synthesis (7).Cysteine is a limiting substrate for de novo GSH synthesis, meaning that external cysteine supply is critical. Therefore, NAC serves as a source of cysteine to overcome acute GSH depletion. While NAC is effective in replenishing GSH levels during GSH deficiency, it is generally ineffective in increasing GSH levels under normal physiological conditions (26). Glutathione biosynthesis is regulated by negative feedback mechanisms, making it difficult to raise GSH levels above physiological levels through NAC supplementation (27)**.**

NAC's anti-inflammatory effects also indirectly contribute to the reduction of oxidative damage. NAC can directly modulate inflammatory signaling pathways, including nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), leading to the suppression of pro-inflammatory cytokines and chemokines. Furthermore, NAC has been shown to inhibit the activation and recruitment of inflammatory cells such as neutrophils and macrophages, thereby reducing tissue inflammation. These combined mechanisms contribute to NAC's overall anti-inflammatory capacity (7).

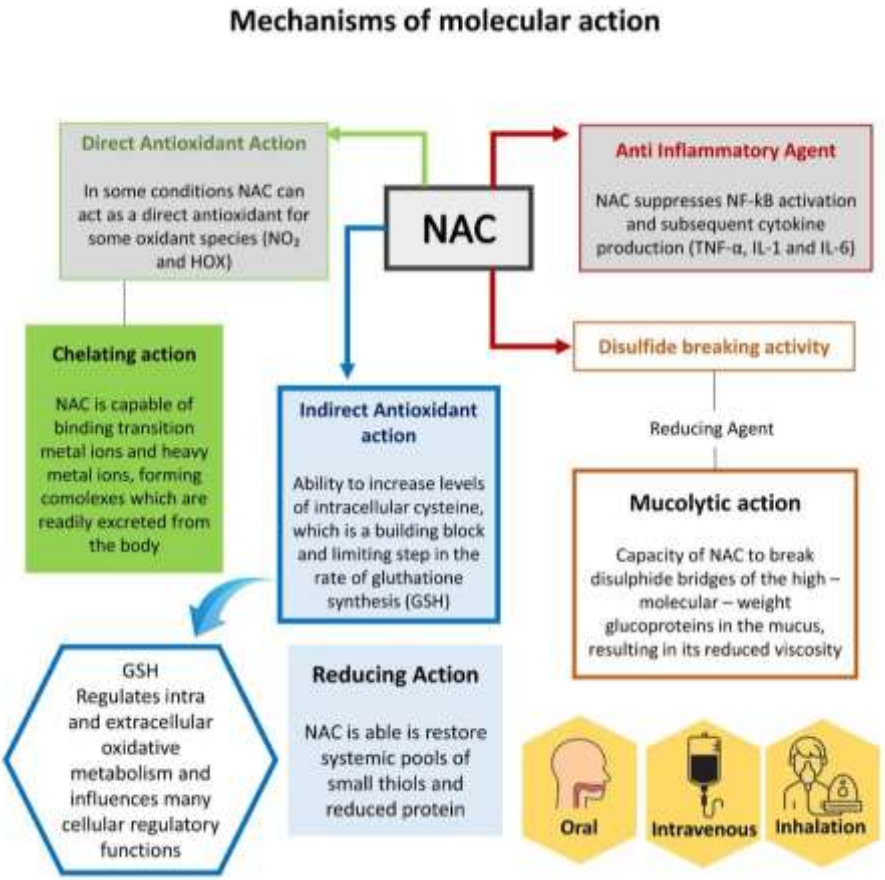

Figure 3. Molecular mechanism of effect or action of N-acetylcysteine (6).

The Role of NAC in Kidney Injury Due to Ischemia-Reperfusion

Based on previous studies, NAC has been proven beneficial for various organs such as the kidneys, muscles, heart, lungs, and liver. Most previous studies have been experimental, examining the effects of NAC using animal models such as rats. Overall, NAC exhibits several effects on the kidneys, such as improving renal blood flow, kidney function, renal tissue damage, and preventing the worsening of oxidative stress and inflammation. Administration of NAC in short-term ischemia can improve renal blood flow (RBF) and increase creatinine clearance by reducing oxidative metabolites (urinary peroxide, nitric oxide, thiobarbituric acid reactive substances/TBARS) and enhancing antioxidant capacity (thiols and glutathione peroxidase) (12).

Other studies show similar results, indicating that NAC treatment prevents renal dysfunction and reduces oxidative stress and inflammation. Kidney functional markers (urea, creatinine) were significantly improved. The activity of antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) was higher, while the levels of oxidants like malondialdehyde (MDA) were lower in the NAC group. Inflammatory markers such as plasma TNF-α and IL-6 were also significantly reduced in the NAC group (13). Another study by Aydin (2020) showed that levels of MDA, myeloperoxidase (MPO), and total nitrite—indicators of oxidative stress—were significantly lower in the kidney IR injury group treated with NAC compared to the placebo group ($p<0.05$). Renal function markers such as urea and creatinine were also found to be lower in the NAC group. These results indicate that NAC has a nephroprotective effect in ischemic kidney injury (28).

Oxidative stress generally arises from enzymatic and non-enzymatic sources, including the xanthine oxidase system, NADPH oxidase system, mitochondrial electron transport chain, and the uncoupled nitric oxide synthase (NOS) system (Wu et al., 2018). However, NAC is known to affect oxidative stress via other pathways. Previous studies have shown that NAC significantly increases the expression of the Nrf2 (Nuclear factor erythroid 2-related factor 2) signaling pathway. The Nrf2 pathway is one of the main adaptive responses activated under oxidative stress conditions. Nrf2 is a transcription factor responsible for inducing phase II detoxifying enzymes whose primary role is to reduce redox stress. These findings support the use of NAC as a novel strategy to protect the kidneys from IR injury (29).

NAC has also been proven to prevent pathological worsening of renal tissue undergoing IR injury. A study tested whether NAC and atorvastatin could prevent kidney tissue damage caused by intestinal ischemia-reperfusion. The results showed that NAC was more effective than atorvastatin in preventing pathological deterioration of the kidneys. NAC was associated with lower pathology scores related to renal necrosis, tubular casts, and interstitial edema, compared to the IR group. The NAC group also showed reduced tubular dilation compared to the IR group (30). Another study evaluating the nephroprotective effects of NAC on progressive kidney injury from prolonged ischemia showed that, based on histopathological analysis, the average pathological score in the NAC group was significantly lower than in the placebo group ($p<0.05$) (28). A study by Sen (2014) evaluated the protective effects of the combined use of dexpanthenol (DXP) and N-acetylcysteine (NAC) in renal ischemia/reperfusion (IR) injury. The combination of NAC and DXP showed better results than either used alone. However, based on histopathology (interstitial edema and inflammation), the NAC group had better outcomes than the DXP group (31).

In addition to animal studies, the protective effects of NAC against IR injury in the kidneys have been tested in humans using randomized controlled trials (RCTs). A double-blind randomized clinical trial was conducted on 50 deceased donor kidney transplant recipients. This study evaluated neutrophil gelatinase-associated lipocalin (NGAL) levels, a promising biomarker for early detection of tubular kidney injury. Patients were randomly assigned to two groups receiving either 600 mg NAC twice daily or a placebo (from day 0 to day 5). The incidence of reduced graft function (RGF) was significantly lower in the NAC group compared to the placebo group (21.4% vs. 50%). Patients in the NAC group experienced a significantly greater reduction in p-NGAL on days 1 and 5 post-transplant compared to the placebo group. Estimated glomerular filtration rate (eGFR) stabilized in the NAC group by week 1, compared to a slower eGFR increase in the placebo group, which stabilized by week 4. NAC shows promising potential in reducing tubular injury and improving graft function, evidenced by a significant decrease in RGF rates and p-NGAL levels (32).

Another RCT study showed different results. This study used NGAL and interleukin-18 (IL-18) as urinary biomarkers for detecting delayed graft function (DGF). It evaluated the protective effects of NAC and its combination with vitamin C on DGF in living donor kidney transplantation. The results showed no significant differences in the prevalence or duration of DGF between the groups. Although NGAL and IL-18 levels decreased in the NAC and NAC + vitamin C groups, these reductions were not significant. Glomerular filtration rate at 30 and 60 days post-transplantation also did not differ significantly between groups. This study suggests that while NAC is a safe drug with no significant side effects for kidney transplant recipients, its potential benefits on DGF biomarkers were not evident (33).

The previously described studies evaluated the protective effects of NAC in acute renal ischemia. However, the effects of NAC in prolonged ischemia have shown different results. A study by Small (2018) evaluated the impact of NAC antioxidants on oxidative stress and mitochondrial function during acute kidney injury (AKI) from ischemia-reperfusion and its

progression to chronic kidney pathology in rats. NAC was found to reduce apoptosis of cortical renal tubular epithelial cells during early IR injury, indicating acute protection. However, prolonged NAC therapy disrupted the renal redox environment and promoted apoptosis in chronically injured kidneys. The increase in TGF-β1 secretion was likely responsible for driving the progression of chronic kidney pathology. The study concluded that NAC's antioxidant effects did not reduce oxidative damage in progressive kidney pathology; instead, they significantly altered the cellular redox environment to promote metabolic and mitochondrial dysfunction. These findings do not support the use of long-term NAC antioxidant therapy for progressive chronic kidney disease (34).

Other studies have also reported similar findings to Small (2018). NAC was found to have significant protective effects in short-term renal ischemia but was ineffective in long-term ischemia. Parameters such as renal blood flow (RBF) and oxidative stress markers did not change after NAC administration in prolonged ischemia. Kidney injury was found to worsen with longer ischemia periods due to likely irreversible damage. Therefore, this study concluded that the duration of ischemia determines the severity of acute kidney injury, and that NAC shows antioxidant effects in short-term ischemia but not in long-term ischemia, emphasizing the possibility of a therapeutic window for its nephroprotective effects (12).

3. CONCLUSION

Ischemia-reperfusion injury results from various pathological mechanisms such as oxidative stress and inflammation. In the kidneys, IR injury can lead to ischemic acute tubular necrosis, as well as epithelial and endothelial damage, which can impair renal function. Tubular cell death due to necrosis and apoptosis is a key mechanism associated with IR injury in the kidneys. Antioxidant therapy, including N-acetylcysteine, is one approach to reduce damage caused by IR injury. NAC has been proven to have nephroprotective effects in IR injury; however, some controversies remain regarding its use in long-term injury. Further research is necessary to enhance understanding of therapeutic approaches in ischemia-reperfusion-induced kidney injury.

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