BRIEF REVIEW

Volatile Anesthetics and AKI: Risks, Mechanisms, and a Potential Therapeutic Window

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ABSTRACT

AKI is a major clinical problem with extremely high mortality and morbidity. Kidney hypoxia or ischemia-reperfusion injury inevitably occurs during surgery involving renal or aortic vascular occlusion and is one of the leading causes of perioperative AKI. Despite the growing incidence and tremendous clinical and financial burden of AKI, there is currently no effective therapy for this condition. The pathophysiology of AKI is orchestrated by renal tubular and endothelial cell necrosis and apoptosis, leukocyte infiltration, and the production and release of proinflammatory cytokines and reactive oxygen species. Effective management strategies require multimodal inhibition of these injury processes. Despite the past theoretical concerns about the nephrotoxic effects of several clinically utilized volatile anesthetics, recent studies suggest that modern halogenated volatile anesthetics induce potent anti-inflammatory, antinecrotic, and antiapoptotic effects that protect against ischemic AKI. Therefore, the renal protective properties of volatile anesthetics may provide clinically useful therapeutic intervention to treat and/or prevent perioperative AKI. In this review, we outline the history of volatile anesthetics and their effect on kidney function, briefly review the studies on volatile anesthetic-induced renal protection, and summarize the basic cellular mechanisms of volatile anesthetic-mediated protection against ischemic AKI.


AKI is a major clinical problem with extremely high mortality and morbidity costing >$10 billion annually in the United States.1,2 AKI is a growing clinical concern because the number of patients who develop AKI has nearly doubled over the past 7 years.3 In hospitalized patients, the incidence of AKI approaches 5%–20%, but it is significantly higher (>36%) in patients who require intensive care unit (ICU) admission and exceeds 60% during the ICU stay.4 Of all cases of AKI in the hospital, approximately 30%–40% occur during the perioperative period.5,6 AKI-related mortality remains extremely high, in which 7%–23% patients die after suffering from uncomplicated AKI and 50%–80% die in the ICU setting.7,8

Renal ischemia-reperfusion (IR) injury is a leading cause of perioperative AKI and frequently complicates major vascular, transplant, cardiac, and liver surgeries.9,10 Although hemodialysis and continuous hemofiltration can acutely treat the symptoms of AKI, allowing the kidney function to recover, significant mortality occurs from AKI as a result of the lack of effective clinical therapy to prevent and/or facilitate the repair of renal cell damage from AKI.11 Furthermore, AKI-induced extrarenal organ injury to the lung, liver, intestine, and brain frequently leads to multiple organ dysfunction syndrome, sepsis, and death.12–14 As the disease progresses, multiple organs are affected, which significantly contributes to morbidity and mortality.7

Tissue injury from surgery or ischemic/hypoxic organ damage creates major stress responses, including autonomic, hormonal, and metabolic changes, and stimulates systemic inflammatory reactions, which are associated with increased postoperative morbidity and mortality. By acting on the brain and on the spinal cord, sufficient clinical anesthetic depth achieved with volatile anesthetics reduces these stress responses.15 However, the invisible nonanesthetic effects of volatile anesthetics in modulating the inflammatory responses have for the most part been ignored and hidden under the shadow of detrimental halogenated anesthetic renal toxicity.16 This brief review provides evidence that halogenated volatile anesthetics are not nephrotoxic as previously believed, but, instead, are renal protective due to their powerful anti-ischemic and anti-inflammatory effects; reviews the protective effects of volatile anesthetics against ischemic AKI in preclinical studies; summarizes the cellular mechanisms of volatile anesthetic-induced kidney protection; and discusses
their potential use to protect kidney function during the perioperative period.

**HISTORICAL VOLATILE ANESTHETICS AND THEIR EFFECT ON RENAL FUNCTION**

Volatile anesthetics are administered to virtually all patients subjected to general anesthesia and are an integral part of the perioperative period. In addition to their analgesic and anesthetic properties, currently utilized halogenated volatile anesthetics are well known to have powerful nonanesthetic properties. For example, several clinically utilized volatile anesthetics (e.g., isoflurane, sevoflurane, and desflurane) have effects on systemic and pulmonary BP, cardiac inotropy, heart rate, and airway smooth muscle tone.17

Since the first successful administration of the general anesthesia with ethyl ether by Morton in 1846,18 significant efforts have been made to develop stable and nonflammable anesthetics. The first nonflammable halogenated volatile anesthetic gas, methoxyflurane, was first synthesized in 1948 by a team of chemists involved in the Manhattan Project during World War II.19 Unfortunately, the clinical use of fluorinated methoxyflurane led to frequent and significant kidney toxicity.20 Methoxyflurane causes vasopressin-resistant high-output renal insufficiency secondary to biotransformation of methoxyflurane to inorganic fluoride by the hepatic cytochrome P450 system.21,22 The inorganic fluoride formation as the cause of volatile anesthetic-induced nephrotoxicity was subsequently generalized to newer fluorinated anesthetics without any sound scientific evidence. Indeed, subsequent animal and human studies demonstrated that neither the peak value of fluoride nor the duration of systemic fluoride exposure correlated with anesthetic nephrotoxicity.23,24 Decades later, another potential concern was raised for clinical use of a widely popular volatile anesthetic, sevoflurane, due to the degradation of sevoflurane by carbon dioxide absorbers (strong alkali). When sevoflurane comes in contact with soda lime absorbers, it undergoes dehydrofluorination to form haloalkenes (called compound A) that have been shown to be severely nephrotoxic in rats.25 Unlike in vivo rat studies, clinical studies indicate that compound A formation during sevoflurane anesthesia has no clinically significant renal effects at any fresh gas flow rate.26 Therefore, the effects of compound A on renal function are not a contemporary clinical concern.

Contrary to the generalized and scientifically unproven historical perception of halogenated anesthetic–induced nephrotoxicity, recent studies show that volatile anesthetics possess powerful multiorgan protective effects during and after ischemic and inflammatory conditions that frequently occur during the perioperative period. It is becoming increasingly clear that volatile anesthetics powerfully modulate IR injury and inflammation in vivo and in vitro.27 In the heart, pretreatment with halothane or isoflurane improves left ventricular systolic function after 15 minutes of left anterior descending coronary artery occlusion.28 Subsequent studies discovered the protective effects of volatile anesthetic pretreatment before prolonged ischemia in other organs (e.g., liver, brain) and this phenomenon was termed anesthetic preconditioning.29,30 Furthermore, volatile anesthetics also protect several organs, including the kidney and heart, when administered after completion of ischemic insult and this phenomenon was termed anesthetic postconditioning.31 These studies establish volatile anesthetics as new potential therapeutic agents to protect against organ inflammation and ischemia.

**VOLATILE ANESTHETICS AND RENAL PROTECTION MECHANISMS**

The cellular mechanisms of ischemic AKI are briefly summarized in Figure 1. Renal IR results in upregulation of several proinflammatory cytokines (e.g., TNF-α), chemokines (e.g., monocyte chemoattractant protein-1, macrophage inflammatory protein-2, and IL-8), and adhesion molecules (e.g., intercellular adhesion molecule-1, vascular cell adhesion molecule-1) in several cell types in the kidney.32 Proinflammatory cytokines are produced in dying or injured proximal tubules and endothelial cells, as well as in infiltrating leukocytes, including neutrophils, macrophages, and lymphocytes.33 In addition to cytokine-induced local inflammation, chemokines attract cytotoxic neutrophils and cytotoxic T lymphocytes to the kidney and contribute to local inflammation after IR injury.33,34 On the other hand, a subset of the T-cell population (regulatory T cells) plays an important role in protecting the kidney from ischemic AKI by suppressing inflammation and facilitating recovery. Regulatory T cells produce multiple anti-inflammatory mediators, including IL-10, TGF-β1, and programmed cell death protein-1, which can counteract the effects of IR injury.35,36 Furthermore, regulatory T cells express ectonucleoside triphosphate diphosphohydrolase (CD39) and ecto-5′-nucleotidase (CD73), which convert proinflammatory ATP to cytotoxic adenosine. Enhanced adenosine generation in turn produces powerful anti-inflammatory effects via A2B adenosine receptors.37

Several studies show that volatile anesthetics have profound protective effects on the kidney by attenuating renal tubular necrosis and decreasing the nephrotoxic effects of proinflammatory leukocyte infiltration and cytokine generation after renal IR injury.16,38,39 Isoflurane or sevoflurane treatment during ischemia and 3-hour reperfusion decreased plasma creatinine by half, with marked improvements in kidney histology and markers of inflammation.16 We also showed that volatile anesthetics decrease the nuclear translocation of NF-κB, a key proinflammatory transcription factor.16 To note, volatile anesthetics promote or produce some of the identical anti-inflammatory mediators involved in renal protection provided by regulatory T cells, including TGF-β1 release, CD73 activation, and adenosine generation (see below).40,41

The detailed mechanisms of action and the exact target location for volatile anesthetics to induce general anesthesia
analgesia, amnesia, immobility) remain ambiguous despite decades of extensive research; however, the lipid membrane is considered a primary site of anesthetic action. This hypothesis is rooted from the striking observations made by Meyer and Overton in 1899, in which the authors noted that anesthetic potencies of 17 different volatile anesthetic agents are linearly related to lipid solubility. This phenomenon was called Meyer and Overton’s rule. Therefore, lipid solubility and its capacity to interact with the lipid membrane bilayer differ among clinically used volatile anesthetics. Interestingly, we determined that desflurane, the least lipid-soluble volatile anesthetic, was less potent in providing renal protection compared with other more lipid-soluble volatile anesthetics, such as sevoflurane, isoflurane, or halothane. Consistent with these findings in the kidney, liver heme oxygenase-1 expression is differentially regulated by several volatile anesthetics; isoflurane and sevoflurane upregulated heme oxygenase-1 mRNA and protein expression, whereas desflurane failed to induce heme oxygenase-1.

In vitro studies in epithelial and endothelial cells suggest that halogenation (fluorinated carbon groups) is responsible for the immunomodulatory effects of volatile anesthetics. The trifluorocarbon (CF3) molecule, which is shared in all newer volatile anesthetics, has been suggested as the specific molecular group of a volatile anesthetic that exerts

Figure 1. Cellular mechanisms of ischemic AKI. Prolonged renal ischemia causes significant depletion of ATP, leading to various cellular changes (e.g., bleb formation and loss of renal tubular polarity). Adhesion molecules and neutrophil chemoattractants expressed on the endothelial cells cause migration of neutrophils. Cytokines and chemokines produced by renal tubular epithelial cells further cause renal tubular and endothelial inflammation. Breakdown of the endothelial basement membrane causes vascular leakage and neutrophil migration into the interstitial space. Orchestration of dendritic cells, neutrophils, and T lymphocytes further promotes epithelial and endothelial injury by inducing inflammation and cytokine/chemokine generation. On the other hand, a subset of the T-cell population, called regulatory T cells, plays an important role in protecting the kidney from IR injury by suppressing inflammation and by facilitating recovery. Volatile anesthetics via release of multiple cytoprotective and anti-inflammatory molecules can target many of the pathways involved in renal tubular, endothelial, and interstitial inflammation and injury.
immunomodulatory effects (Figure 2). In pulmonary epithelial and endothelial cells, modulation of chemical structures of volatile anesthetics showed that the CF₃ molecular group is required for decreasing multiple proinflammatory cytokine markers after LPS treatment. Diethyl ether or structure-similar nonfluorinated molecules failed to produce anti-inflammatory effects.

The mechanisms of volatile anesthetic-mediated protection against ischemic AKI are most likely different from cardiac IR models despite the similar anti-inflammatory effects produced. Whereas volatile anesthetics protect the heart via activation of ATP-dependent potassium (K⁺_ATP) channels, kidney protection mechanism involves multiple signaling pathways including TGF-β1 generation, sphingosine kinase (SK) activation, adenosine generation, and IL-11 synthesis independent of the K⁺_ATP channels. In addition, volatile anesthetics must be present during renal ischemia to provide protection, whereas pretreatment alone is protective against cardiac IR injury.

Volatile Anesthetics Release Renal Tubular TGF-β1 and Promote Plasma Membrane Caveolae Formation
Disruption of the plasma membrane lipid bilayer by volatile anesthetics results in the translocation of phosphatidylserine from the inner leaflet to the outer leaflet of the plasma membrane (Figure 3). Phosphatidylserine (PS) externalization in macrophages as well as in renal tubular epithelia leads to the release of a potent anti-inflammatory/antinecrotic molecule TGF-β1 in adjacent cells via ligation of PS receptors. Indeed, TGF-β1 has been recognized as a key signaling molecule in the anti-inflammatory milieu of macrophages ingesting apoptotic cells. We demonstrated that the release of TGF-β1 is critically involved in generating the anti-inflammatory and antinecrotic effects of volatile anesthetics to protect against ischemic AKI in mice. TGF-β1-deficient mice were not protected against renal IR injury with sevoflurane anesthesia. Furthermore, a neutralizing TGF-β1 antibody blocked renal protection with sevoflurane in wild-type mice.

In addition, sevoflurane caused nuclear translocation of the mothers against decapentaplegic homolog 3 (SMAD-3) transcription factor in primary cultures of proximal tubules from wild-type mice but not in proximal tubules from TGF-β1-deficient mice. Furthermore, SMAD-3-deficient mice were not protected against renal IR injury with sevoflurane anesthesia. Finally, isoflurane-induced PS externalization also causes an increase in caveolae/caveolin lipid rafts in the buoyant fractions of the plasma membranes, and an increase in caveolae sequestration of several key signaling intermediates, which are critical for volatile anesthetic-mediated renal protection including TGF-β1 receptors, as well as SKs, extracellular signal-regulated kinases, and sphingosine-1-phosphate (S1P) (Figure 3).

Volatile Anesthetics Induce Renal Tubular SK-1 and S1P Synthesis
Most volatile anesthetics are lipophilic molecules and can activate sphingomyelin hydrolysis. The membrane-bound lysophospholipid receptor for S1P is one of the molecules sequestrated in the plasma membrane caveolae. Lyso-phospholipid S1P is a product of sphingomyelin hydrolysis by SK and functions as both an extracellular ligand for specific G protein-coupled receptors, as well as an intracellular second messenger. S1P promotes cell growth and survival and regulates lymphocyte egress and migration. S1P binds to five subtypes of specific G protein-coupled receptors and mediates its antiapoptotic effects via pathways involving Akt and extracellular signal-regulated kinase signaling in hepatic myofibroblasts, lung epithelium, and melanocytes. We have shown that SK and S1P signaling play a major role in volatile anesthetic-induced protection against renal IR injury. Clinically used volatile anesthetic isoflurane-induced S1P synthesis via induction of SK-1 synthesis in renal proximal tubules and cultured endothelial cells. In addition, S1P receptor blockade by selective S1P receptor antagonists reversed the isoflurane-mediated renal protection. The administration of SK-1 inhibitors also reversed the
isoflurane-mediated protection against renal IR injury. In addition, mice lacking the SK-1 enzyme were not protected against renal IR injury with isoflurane.

**Volatile Anesthetics Induce Renal Tubular Adenosine Synthesis via Activation of CD73**

It is becoming increasingly clear that volatile anesthetic–mediated TGF-β1 release activates additional cytoprotective pathways. We recently demonstrated that isoflurane–mediated release of TGF-β1 induces CD73 synthesis in vivo as well as in vitro, leading to increased renal tubular adenosine generation. Isoflurane-mediated induction of CD73 and adenosine generation was critical for protection against ischemic AKI in mice. A selective CD73 inhibitor or CD73 deficiency prevented the renal protective effect of isoflurane anesthesia. Cell-surface CD73 catalyzes the hydrolysis of AMP to generate adenosine and is considered the rate-limiting step in extracellular adenosine generation. Adenosine signaling regulates diverse physiologic effects, including cardiovascular control, tissue injury, and inflammation in many organs by binding to four subtypes of...
adrenosine receptors (ARs).\textsuperscript{65–68} Indeed, CD73 activation and adenosine generation protect against renal, intestinal, and cardiac IR injury by reducing necrosis and inflammation.\textsuperscript{69–71} In particular, renal tubular A1AR activation reduces necrosis and apoptosis, whereas A2aAR stimulation leads to tissue protection by attenuating leukocyte-mediated inflammation after renal IR. Renal A1ARs dramatically reduce proximal tubular necrosis and apoptosis via activation of extracellular signal–regulated kinase, Akt, and heat shock protein 27 through a peritubular signal mediated by A1AR-mediated renal tubular


Volatile Anesthetics Induce Renal Tubular IL-11 Synthesis via A1AR Activation

Volatile anesthetic therapy for critically ill patients may be limited by its anesthetic and hemodynamic effects. One way to mitigate this is to utilize the distal signaling molecules synthesized with volatile anesthetic treatment devoid of systemic hemodynamic and anesthetic effects. Volatile anesthetic isoflurane-mediated induction of CD73 activation and adenosine generation subsequently induces IL-11 expression in human renal proximal tubular cells and in mouse kidney.\textsuperscript{72,73} We recently showed that isoflurane-mediated protection against ischemic AKI is additionally mediated by A1AR-mediated renal tubular IL-11 synthesis and release.\textsuperscript{74} IL-11 is a 20-kD member of the IL-6–type cytokine family. IL-11 promotes megakaryocyte maturation\textsuperscript{76} and is already clinically approved to treat severe thrombocytopenia in patients receiving chemotherapy.\textsuperscript{77} In addition to its hematopoietic effects, IL-11 protects against intestinal, cardiomyocyte, and endothelial cell death.\textsuperscript{78} We also recently showed that recombiant human IL-11 treatment before or after renal ischemia attenuated ischemic AKI in mice. Specifically, IL-11 administration significantly attenuated necrosis, inflammation, and apoptosis after ischemic AKI closely mimicking the renal protective effects of volatile anesthetics. Furthermore, this IL-11-mediated protection against ischemic AKI also requires the downstream induction of SK-1, another cytoprotective protein.\textsuperscript{79}

Renal IR injury results in intensive tissue hypoxia with subsequent hypoxia-inducible factor (HIF) stabilization, and HIF-1α–dependent transcriptional regulation plays a critical role in modulating tissue hypoxia and inflammation as well as promoting cellular repair.\textsuperscript{80,81} Interestingly, HIF-1α signaling is critical for IL-11–mediated protection against ischemic AKI.\textsuperscript{82} Moreover, ischemia or hypoxia promotes HIF-1α–dependent transcriptional regulation of adenosine-generating enzymes (CD39 or CD73)\textsuperscript{80} that can directly stimulate IL-11 synthesis.\textsuperscript{79} Previous studies also suggest that a widely used volatile anesthetic, isoflurane, increases HIF-1α expression in a renal carcinoma cell line and in the rabbit myocardium.\textsuperscript{83,84} Finally, a recent study showed that isoflurane failed to protect mice pretreated with HIF-1α small interfering RNA, suggesting a critical role for HIF-1α signaling in isoflurane-mediated protection against ischemic AKI.\textsuperscript{85}

CLINICAL EVIDENCE FOR VOLATILE ANESTHETIC–MEDIATED ORGAN PROTECTION

Current American Heart Association guidelines recommend the use of volatile anesthetics for maintenance of general anesthesia during noncardiac surgery in high-risk and hemodynamically stable patients at risk for perioperative myocardial ischemia (class IIb evidence).\textsuperscript{86} In 64 patients subjected to liver resection, sevoflurane preconditioning significantly decreased postoperative elevation of transaminase levels.\textsuperscript{87} In terms of renal protection, in 72 patients subjected to coronary artery bypass graft surgery, 10 minutes of sevoflurane preconditioning not only significantly decreased the release of brain natriuretic peptide, a biochemical marker of myocardial contractile dysfunction, but also significantly reduced postoperative plasma cystatin C concentrations, suggesting improvements in cardiac and renal function after major heart surgery.\textsuperscript{88}

It is clear that more randomized clinical studies are needed to better define the renal protective role for volatile anesthetics against human AKI. There are currently several prospective surgical trials registered in ClinicalTrials.gov comparing the renal outcome after anesthesia with volatile anesthetics or with intravenous anesthetics. Preliminary findings from one of these studies suggest that eGFR improved during the early postoperative period after living donor kidney transplant surgery when the kidney donors were anesthetized with a volatile anesthetic compared with propofol.\textsuperscript{89} One of the major challenges in assessing renal protective effects of volatile anesthetics is lack of a sensitive biomarker to detect early efficacy of volatile anesthetic–mediated protection against AKI. Currently, clinically utilized markers (e.g., plasma creatinine, eGFR) are insensitive to truly test the efficacy of renal protection.

FUTURE IMPLICATIONS AND CHALLENGES

Despite the growing incidence of AKI, there is currently no effective clinical therapy for AKI.\textsuperscript{11} Harnessing the anti-inflammatory and anti-ischemic effects of clinically utilized volatile anesthetics would be an excellent therapy to mitigate the detrimental effects of AKI. Volatile anesthetics activate multiple pathways to synthesize several key cytoprotective and anti-inflammatory signaling molecules to attenuate ischemic AKI, including TGF-β1 release, caveolae organization of SK and S1P signaling, CD73 activation,
adrenosine synthesis, and IL-11 generation (Figure 3). The optimal therapeutic window, method of delivery, dose, and exposure time of volatile anesthetics for renal protection need to be further elucidated. In addition, the molecular mechanisms of volatile anesthetics including lipid interactions and protein kinases involved in the downstream effects of anesthetic-induced renal protection need to be further studied. Finally, clinical introduction of a sensitive biomarker to detect AKI is critically needed to better assess the early efficacy of volatile anesthetic–mediated protection against ischemic AKI. New therapeutic use of volatile anesthetics will facilitate their off-label use as adjuncts for kidney protection during the perioperative period. In summary, harnessing the nonanesthetic properties of volatile anesthetics may have important clinical implications for critically ill patients anesthetized in the operating room and sequestered in the ICU.

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DISCLOSURES

None.

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