Critical Role of Interleukin-11 in Isoflurane-mediated Protection against Ischemic Acute Kidney Injury in Mice

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ABSTRACT

Background: Isoflurane releases renal tubular transforming growth factor-β1 (TGF-β1) and protects against ischemic acute kidney injury. Recent studies suggest that TGF-β1 can induce a cytoprotective cytokine interleukin (IL)-11. In this study, the authors tested the hypothesis that isoflurane protects against ischemic acute kidney injury by direct induction of renal tubular IL-11 synthesis.

Methods: Human kidney proximal tubule cells were treated with 1.25–2.5% isoflurane or carrier gas (room air + 5% carbon dioxide) for 0–16 h. The authors also anesthetized C57BL/6 mice with 1.2% isoflurane or with equianesthetic dose of pentobarbital for 4 h. In addition, the authors subjected IL-11 receptor (IL-11R) wild-type, IL-11R–deficient, or IL-11 neutralized mice to 30-min renal ischemia followed by reperfusion under 4 h of anesthesia with pentobarbital or isoflurane (1.2%).

Results: Isoflurane increased IL-11 synthesis in human (approximately 300–500% increase, N = 6) and mouse (23 ± 4 [mean ± SD] fold over carrier gas group, N = 4) proximal tubule cells that were attenuated by a TGF-β1–neutralizing antibody. Mice anesthetized with isoflurane showed significantly increased kidney IL-11 messenger RNA (13.8 ± 2 fold over carrier gas group, N = 4) and protein (31 ± 9 vs. 18 ± 2 pg/mg protein or approximately 80% increase, N = 4) expression compared with pentobarbital-anesthetized mice, and this increase was also attenuated by a TGF-β1–neutralizing antibody. Furthermore, isoflurane-mediated renal protection in IL-11R–deficient mice was absent in IL-11R–deficient mice or in IL-11R wild-type mice treated with IL-11–neutralizing antibody (N = 4–6).

Conclusion: In this study, the authors suggest that isoflurane induces renal tubular IL-11 via TGF-β1 signaling to protect against ischemic acute kidney injury.

What We Already Know about This Topic

- Halogenated anesthetics protects against acute kidney injury by production of renal tubular transforming growth factor-β1

What This Article Tells Us That Is New

- Isoflurane increased interleukin-11 synthesis in human and mouse proximal tubular cells via transforming growth factor-β1 signaling to protect against ischemic acute kidney injury.
mechanisms of volatile halogenated anesthetic-mediated renal protection generated by TGF-β1 remain incompletely understood. Moreover, isoflurane therapy for critically ill patients may be limited by its anesthetic and cardiovascular effects. One way to mitigate this is to use the distal signaling molecules synthesized with isoflurane treatment devoid of systemic hemodynamic and anesthetic effects.

Interleukin (IL)-11 is a 20 kDa member of the IL-6-type cytokine family. IL-11 promotes megakaryocyte maturation and is already clinically approved to increase platelet counts in patients receiving chemotherapy. In addition to its hematopoietic effects, IL-11 protects against intestinal, cardiomyocyte, and endothelial cell death. We recently showed that recombinant 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 halogenated anesthetics. This IL-11-mediated protection against ischemic AKI requires the downstream induction of another cytoprotective protein sphingosine kinase-1. Interestingly, we also showed that isoflurane-mediated protection against ischemic AKI also requires induction of sphingosine kinase-1. Finally, previous studies suggest that TGF-β1 induces IL-11 in lung epithelial cells and fibroblasts. Therefore, in this study, we tested the hypothesis that isoflurane induces TGF-β1-mediated renal proximal tubular IL-11 synthesis. We also tested whether IL-11 plays a critical role in isoflurane-mediated renal protection.

Materials and Methods

Human and Mouse Proximal Tubule Cell Culture and Exposure to Isoflurane

Immortalized human renal proximal tubule (HK-2) cells (American Type Culture Collection, Manassas, VA) were grown and passaged with 50:50 mixture of Dulbecco Modified Eagle Media/F12 with 10% fetal bovine serum (Invitro-gene, Carlsbad, CA) and antibiotics (100 μg/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B; Invitrogen) at 37°C in a 100% humidified atmosphere of 5% carbon dioxide–95% air. This cell line has been characterized extensively and retains the phenotypic and functional characteristics of proximal tubule cells in culture. We also cultured mouse kidney proximal tubule cells. Mouse kidneys were removed, minced, and digested in collagenase A (1 mg/ml; Sigma, St. Louis, MO) at 37°C for 45 min with occasional agitation. The cellular digest was filtered through a nylon mesh, centrifuged at 600g for 10 min, and washed twice. Mouse kidney proximal tubules were isolated according to the method by Vinay et al. with the use of Percoll density gradient separation. Cells were used in the experiments described below when confluent after 24-h serum deprivation.

HK-2 cells or mouse proximal tubules in culture were placed in an air tight, 37°C, humidified modular incubator chamber (Billups-Rothenberg, Inc., Del Mar, CA) with inflow and outflow ports. The inlet port was connected to a vaporizer (Datex-Ohmeda, GE Healthcare, Oklahoma City, OK) to deliver isoflurane (Abbott Laboratories, North Chicago, IL) mixed with 95% air and 5% carbon dioxide (carrier gas) at 10 l/min. The outlet port was connected to a Datex-Ohmeda 5250 RGM gas analyzer that measured isoflurane concentrations. Exposure to isoflurane (1.25–2.5%) lasted 0–16 h. Control cells were exposed to carrier gas in an identical modular incubator chamber. To block the effects of TGF-β1 generated by isoflurane, some HK-2 cells were pretreated with neutralizing TGF-β1 antibody (10 μg/ml; R&D Systems, Minneapolis, MN) 30 min before isoflurane treatment, respectively. We also used nonneutralizing control isotype antibody to test the specificity of the neutralizing TGF-β1 antibody (BD Biosciences, San Jose, CA).

Reverse Transcription Polymerase Chain Reaction for IL-11

By using reverse transcription polymerase chain reaction, we measured messenger RNAs (mRNAs)-encoding human (HK-2 cells) or mouse IL-11 as described. Amplification of the human IL-11 complementary DNA was performed using the following primers: forward primer, 5′-CTG TGA CC-3′ and reverse primer, 5′-CAG GCC AGA AGT CTG TGG AC-3′ at an annealing temperature of 66°C resulting in a 300 base pair product. Amplification of the mouse IL-11 complementary DNA was performed using the following primers: forward primer, 5′-AAC TGT GTT CGC CTG GTG-3′ and reverse primer, 5′-AAG CTG CAA AGA CAA TG-3′ at an annealing temperature of 68°C resulting in a 267 base pair product. Glyceraldehyde-3-phosphate dehydrogenase complementary DNA amplification was performed to control for lane loading: forward primer, 5′-ACC ACA GTG CAT GCC ATC AC-3′ and reverse primer, 5′-CAC CAC CCT GTT GCT GTA GCC-3′ at an annealing temperature of 65°C resulting in a 450 base pair product.

IL-11 Enzyme-linked Immunosorbent Assay

HK-2 cell supernatant or mouse kidney cortex lysate IL-11 protein expression was measured using human- or mouse-specific sandwich IL-11 enzyme-linked immunosorbent assay kit (R&D Systems), respectively.

Mouse Anesthesia and Induction of Renal I/R Injury

After approval from the Columbia University Institutional Animal Care and Use Committee (New York, New York), we used adult male IL-11 receptor–deficient (IL-11R knockout) mice or wild-type (WT) litter mates (IL-11R WT) on a C57BL/6 background (B6.129S1-Ill1r1m1WtWh/J; Jackson Labs, Bar Harbor, ME). Mice were initially anesthetized with intraperitoneal pentobarbital (Henry Schein Veterinary Co., Indianapolis, IN; 50 mg/kg body weight, or to effect) and subjected to right nephrectomy and 30 min of left renal ischemia or to sham-operation (laparotomy; right nephrectomy without renal ischemia). After closure of the abdomen in two layers, the mice were then exposed to an additional 4 h of equipotent doses of either pentobarbital or
1.2% isoflurane as described previously. The mice were placed on a heating pad under a warming light to maintain body temperature approximately 36°–38°C. To neutralize IL-11 in vivo, some IL-11R WT mice were injected intravenously with 1 mg/kg monoclonal anti-IL-11 (MAB418; R&D Systems) 20 min before reperfusion of ischemic kidney or sham-operation. We collected kidney (cortex and corticomedullary junction) and plasma 6–24 h after I/R injury to examine the severity of renal...

Fig. 1. Isoflurane induces interleukin (IL)-11 messenger RNA (mRNA) and protein synthesis in human kidney proximal tubule (HK-2) cells. (A and B) IL-11 mRNA measured by reverse transcription polymerase chain reaction in HK-2 cells treated with 0–2.5% isoflurane for 6 h (A; N = 6) or 2.5% isoflurane for 0–6 h (B; N = 6). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression was quantified to normalize lane loading. Data are presented as means ± SD. *P < 0.05 versus IL-11 mRNA measured after 0% isoflurane treatment (A) or at 0 h (B). (C) Isoflurane increases IL-11 protein (pg/ml) in cell culture media from HK-2 cells (N = 6). HK-2 cells were treated with 2.5% isoflurane or with carrier gas for 6 or 16 h. *P < 0.05 versus carrier gas–treated group.

Fig. 2. Isoflurane induces interleukin (IL)-11 in human kidney proximal tubule (HK-2) cells via transforming growth factor-β1 (TGF-β1). (A) IL-11 messenger RNA (mRNA) (detected by reverse transcription polymerase chain reaction) expression in HK-2 cells treated with 2.5% isoflurane for 6 h (N = 6). Representative images (top) and band intensity quantifications (bottom) expressed as fold increases in IL-11 expression over carrier gas plus immunoglobulin G (IgG) isotype antibody–treated controls. (B) IL-11 protein (detected with enzyme-linked immunosorbent assay) expression in HK-2 cells treated with 2.5% isoflurane for 6 h (N = 6). *P < 0.05 versus carrier gas group treated with IgG isotype antibody. #P < 0.05 versus isoflurane group treated with IgG isotype antibody. Error bars represent 1 SD. TGF-β1 antibody (10 μg/ml) prevents isoflurane-mediated induction of IL-11 mRNA and protein expression in human proximal tubule cells.
dysfunction (plasma creatinine, renal histology, apoptosis, and neutrophil infiltration) and IL-11 mRNA and protein detection. Plasma creatinine was measured as described with an enzymatic creatinine reagent kit according to the manufacturer’s instructions provided by Thermo Fisher Scientific, Waltham, MA.24 Unlike the Jaffe method, this method of creatinine measurement largely eliminates the interferences from mouse plasma chromagens.

Some IL-11R WT mice were anesthetized with pentobarbital or with 1.2% isoflurane without being subjected to renal I/R injury. To test the critical role of TGF-β1 signaling in isoflurane-mediated IL-11 induction in vivo, some IL-11 WT mice were injected intravenously with 5 mg/kg monoclonal anti-TGF-β1 (MAB240; R&D Systems) or control isotype antibody 30 min before anesthesia with isoflurane or pentobarbital.

**Histological Detection of Kidney Necrosis, Apoptosis, and Neutrophil Infiltration**

Morphological assessment of kidney hemotoxylin and eosin stain staining was performed by renal pathologist (Dr. D’Agati) who was unaware of the treatment that each animal had received. An established grading scale of necrotic injury (0–4, renal injury score) to the proximal tubules was used for the histopathological assessment of I/R-induced damage as outlined by Jablonski et al.25 and as described previously in our studies.26–27 We detected kidney apoptosis with terminal deoxynucleotidyl transferase 2′-deoxyuridine-5′-triphosphate nick end-labeling staining as described elsewhere28 using a commercially available in situ cell death detection kit (Roche, Indianapolis, IN) according to the instructions provided by the manufacturer. Apoptotic terminal deoxynucleotidyl transferase 2′-deoxyuridine-5′-triphosphate nick end-labeling–positive cells were counted in five to seven randomly chosen ×100 microscope image fields in the corticomedullary junction, and results were expressed as apoptotic cells counted per ×100 field. Immunohistochemistry for neutrophils was performed as described previously29 with a rat anti-mouse Ly6B monoclonal antibody against polymorphonuclear leukocytes (clone 7/4; AbD Serotec, Raleigh, NC). A primary antibody that recognized IgG2a (MCA1212; AbD Serotec) was used as a negative isotype control in all experiments. Neutrophils infiltrating the kidney were quantified in five to seven randomly chosen ×200 microscope image fields in the corticomedullary junction, and results were expressed as neutrophils counted per ×200 field.

**IL-11 Immunohistochemistry**

Immunohistochemistry detected mouse kidney IL-11 protein expression and localization 4 h after anesthesia with pentobarbital or 1.2% isoflurane with rat anti-IL-11 antibody (MAB418; 1:50 dilution; R&D Systems) and biotin-conjugated anti-rat IgG (1:100 dilution; Vector Laboratories, Burlingame, CA). Normal rat IgG2a (Vector Laboratories) was used at the same concentration as the primary antibody as a negative isotype control. Kidney IL-11 immunohistochemistry was quantified as described by Matkowskij et al.30 with some modifications. Integrated image densities of five to seven randomly selected renal tubule areas from each slide were averaged, and background measured from isotype control slides was subtracted. Renal tubular IL-11 intensity was expressed as fold increase over pentobarbital-anesthetized mice.

**Statistical Analysis**

The data were analyzed with two-tailed Student *t* test when comparing means between two groups or one-way or two-way ANOVA plus Tukey *post hoc* multiple comparison test when comparing multiple groups. The ordinal values of the renal injury scores were analyzed by the Mann–Whitney nonparametric test. We used Graphad InStat and Graphad.
Prism for statistical analyses (GraphPad Software, Inc., La Jolla, CA). In all cases, a probability statistic P value less than 0.05 was taken to indicate significance. All data are expressed throughout the text as means ± SD.

**Results**

**Isoflurane Induces IL-11 in Human and Mouse Proximal Tubule Cells via TGF-β1 Signaling**

Figure 1A shows a concentration-dependent (0–2.5%) induction of IL-11 mRNA in HK-2 cells after isoflurane treatment (N = 6). Figure 1B shows that 2.5% isoflurane significantly increased IL-11 mRNA after 3- or 6-h treatment. Isoflurane treatment (2.5%) in HK-2 cells for 6–16 h also induced IL-11 protein (released into cell culture media; fig. 1C) in HK-2 cells compared with carrier gas–treated cells (N = 6).

HK-2 cells pretreated with control isotype antibody (mouse IgG) demonstrated a significant induction of IL-11 mRNA (fig. 2A) and protein (fig. 2B) expression (N = 6) after isoflurane exposure (2.5% for 6 h). We determined that TGF-β1-neutralizing antibody (10 µg/ml) significantly attenuated the up-regulation of IL-11 mRNA as well as protein expression after isoflurane treatment.

Figure 3 shows that primary cultures of mouse kidney proximal tubule cells pretreated with control isotype mouse IgG had significant IL-11 mRNA induction after 6-h treatment with 2.5% isoflurane (N = 4). TGF-β1 neutralization significantly attenuated the up-regulation of IL-11 mRNA in isoflurane-treated mouse proximal tubule cells. Therefore, our studies show that isoflurane-induced TGF-β1 directly promotes the synthesis of IL-11 in both immortalized and primary cultures of renal proximal tubule cells.

**Isoflurane-mediated Induction of Kidney IL-11 In Vivo via TGFβ1 Signaling**

Figure 4 shows that anesthesia with isoflurane (1.2% for 4 h) significantly induced IL-11 mRNA (fig. 4A) and protein expression (fig. 4B) measured in mouse kidneys compared with mice anesthetized with pentobarbital for 4 h (N = 4). We also determined that TGF-β1–neutralizing antibody (5 mg/kg monoclonal anti-TGF-β1, MAB240) prevented the induction of kidney IL-11 mRNA and protein expression after anesthesia with isoflurane. * P < 0.05 versus pentobarbital-anesthetized mice treated with immunoglobulin G (IgG) isotype antibody. # P < 0.05 versus isoflurane-anesthetized mice treated with IgG isotype antibody. Lines represent means of scatter plots. Anesthesia with isoflurane significantly increased kidney IL-11 mRNA and protein expression in mice. ISO = isoflurane; PB = pentobarbital.
Isoflurane for 4 h (representative of four experiments, ×100 and ×400 images shown). Quantification of immunohistochemical staining confirmed significant increase in IL-11 immunoreactivity in mice anesthetized with isoflurane (fig. 5B; N = 4). This increase in IL-11 immunoreactivity during anesthesia with isoflurane was again attenuated by treating mice with TGF-β1–neutralizing antibody before anesthesia with isoflurane. Therefore, from these experiments, we concluded that isoflurane induces IL-11 and synthesis in vivo via TGF-β1 signaling. IL-11 staining was not visible in the kidneys stained with negative isotype control antibody.

**Critical Role of IL-11 in Isoflurane-mediated Protection against Ischemic AKI In Vivo**

IL-11R WT mice anesthetized with pentobarbital or with 1.2% isoflurane for 4 h had similar plasma creatinine values after sham-operation (fig. 6). Similarly, IL-11R WT mice pretreated with IL-11–neutralizing antibody or IL-11R knockout mice had similar plasma creatinine values after sham-operation during anesthesia with pentobarbital. Plasma creatinine significantly increased in IL-11R WT mice subjected to 30 min of renal ischemia and 2-h reperfusion compared with sham-operated mice (fig. 6; N = 6). However, IL-11R WT mice anesthetized with 1.2% isoflurane for 4 h after renal ischemia (isoflurane postconditioning) had significantly decreased plasma creatinine 24 h after injury compared with mice anesthetized with pentobarbital after renal ischemia. Supporting a critical role of IL-11 in isoflurane-mediated protection against ischemic AKI, IL-11R knockout or IL-11R WT mice pretreated with IL-11–neutralizing antibody before renal ischemia were not protected against ischemic AKI during anesthesia with isoflurane after renal I/R (fig. 6). IL-11R knockout mice and IL-11R WT mice pretreated with IL-11–neutralizing antibody had
IL-11 Is Critical for Isoflurane-mediated Reduction in Kidney Neutrophil Infiltration and Apoptosis

Figure 8A shows representative images (from four to six experiments) of neutrophil immunohistochemistry in kidneys (magnification ×200) of mice subjected to 30 min of renal ischemia and 24-h reperfusion. In sham-operated mice, we were unable to detect any neutrophils in the kidney (data not shown). There was heavy neutrophil infiltration (dark brown) in the kidneys of pentobarbital-anesthetized IL-11R WT mice subjected to renal I/R. In contrast, IL-11R WT mice anesthetized with isoflurane for 4 h after renal ischemia had significantly reduced neutrophil infiltration in the kidney 24 h after I/R (fig. 8B). Again, isoflurane failed to reduce kidney neutrophil infiltration in IL-11R WT mice treated with IL-11-neutralizing antibody and in IL-11R knockout mice.

Terminal deoxynucleotidyl transferase 2′-deoxyuridine-5′-triphosphate nick end-labeling staining (from four to five experiments) detected apoptotic renal cells in the kidneys of mice subjected to renal I/R resulting in severe proximal tubule cell apoptosis (fig. 9A, ×100). Renal ischemia and 24 h of reperfusion resulted in significant apoptosis in the kidneys of pentobarbital-anesthetized IL-11R WT mice. However, IL-11R WT mice anesthetized with isoflurane for 4 h after renal ischemia had significantly reduced number of apoptotic terminal deoxynucleotidyl transferase 2′-deoxyuridine-5′-triphosphate nick end-labeling–positive cells in the kidney 24 h after I/R (fig. 9B). Again, isoflurane failed to reduce renal tubular apoptosis in IL-11R WT mice treated with IL-11-neutralizing antibody and in IL-11R knockout mice.

Discussion

We have shown in this study that isoflurane increases renal tubular IL-11 mRNA and protein synthesis via TGF-β1-dependent signaling in vivo as well as in vitro. TGF-β1 is a powerful antinflammatory cytokine that regulates multiple cellular processes including immune modulation, cellular differentiation and proliferation, and oncogenesis. We previously demonstrated that volatile halogenated anesthetics including isoflurane cause translocation of phosphatidylserine from the inner leaflet to the outer leaflet of the plasma membrane (membrane externalization) resulting in the release of antinflammatory TGF-β1. Furthermore, volatile halogenated anesthetics-mediated reduction in renal tubular necrosis and inflammation was dependent on the release of renal tubular TGF-β1. Finally, volatile halogenated anesthetic-mediated renal protection was abolished in mice deficient in TGF-β1 or WT mice treated with neutralizing TGF-β1 antibody.

We also recently demonstrated that exogenous human recombinant IL-11 attenuated ischemic AKI in mice and reduced necrosis and apoptosis in human kidney (HK-2) proximal tubule cells. In mice, recombinant IL-11 treatment attenuated renal tubular necrosis and apoptosis as

Fig. 6. Interleukin (IL)-11 is critical for isoflurane-mediated renal protection against ischemic acute kidney injury. Plasma creatinine levels from IL-11 wild-type (WT) or IL-11–deficient (knockout [KO]) mice subjected to 30-min renal ischemia and 24-h reperfusion (N = 4–6 per group). After renal ischemia reperfusion (RIR), mice were further anesthetized with 1.2% isoflurane or with equianesthetic dose of pentobarbital. Some IL-11 receptor (IL-11R) WT mice were pretreated with an IL-11–neutralizing antibody (1 mg/kg, intravenous injection) 20 min before reperfusion or sham-operation. Isoflurane postconditioning significantly reduced plasma creatinine after RIR injury in IL-11R WT mice. However, IL-11R deficiency or IL-11–neutralizing antibody prevented the renal protective effects of isoflurane postconditioning. * P < 0.05 versus respective sham-operated mice. # P < 0.05 versus pentobarbital-anesthetized mice subjected to RIR. Data are from six mice per group and represented as mean ± SD. ISO = isoflurane; PB = pentobarbital.

similar degree of renal injury after I/R during anesthesia with pentobarbital. Collectively, these studies suggest that IL-11 induction by isoflurane is required to trigger in vivo renal protection.

Figure 7A demonstrates severe necrotic renal injury in IL-11R WT mice subjected to renal I/R during anesthesia with pentobarbital. Compared with sham-operated mice (not shown), the kidneys of mice subjected to renal I/R showed significant tubular necrosis, cast formation, and congestion. In contrast, consistent with the plasma creatinine data, anesthesia with isoflurane reduced renal tubular necrosis 24 h after I/R injury (fig. 7A). Supporting a critical role of IL-11 in isoflurane-mediated renal protection against I/R, isoflurane failed to reduce renal tubular necrosis in IL-11R WT mice pretreated with a neutralizing IL-11 antibody or in IL-11R knockout mice. The Jablonski scale ischemic renal injury score was used to grade renal tubular necrosis 24 h after renal I/R (fig. 7B). Thirty minutes of renal ischemia and 24 h of reperfusion resulted in severe acute tubular necrosis in IL-11R WT mice anesthetized with pentobarbital after renal I/R injury. Consistent with the renal histology data, isoflurane significantly reduced the renal injury score in IL-11R WT mice but not in IL-11R knockout mice or in IL-11R WT mice pretreated with IL-11–neutralizing antibody.
Isoflurane Induces Renal Protective IL-11

Ham et al.

Isoflurane Induces Renal Protective IL-11

well as the influx of proinflammatory neutrophils after renal I/R. We further demonstrated in this study that isoflurane-mediated protection against renal tubular necrosis (Jablonski score), inflammation (neutrophil infiltration), and apoptosis (terminal deoxynucleotidyl transferase 2’-deoxyuridine-5’-triphosphate nick end-labeling staining) is directly mediated by the induction of IL-11 as isoflurane failed to reduce these critical indices of renal injury in IL-11R–deficient mice or mice treated with IL-11–neutralized antibody. Taken together, as blockade or genetic deletion of either TGF-β1 or IL-11 abolished renal protective effects of isoflurane, our current and previous studies suggest that isoflurane induces nonredundant proximal (TGF-β1) and distal (IL-11) cytoprotective signaling molecules to protect against ischemic AKI.

The most exciting aspect of isoflurane-mediated induction of IL-11 leading to renal protection is that recombinant IL-11 (Oprelvekin; Wyeth Pharmaceuticals, Philadelphia, PA) is already clinically approved to treat chemotherapy-induced thrombocytopenia. IL-11 directly reduces necrosis as well as apoptotic cell death via mitogen-activated protein kinase and the Janus kinase/signal transducers and activators of transcription signaling. In addition to its antiapoptotic and antinecrotic properties, IL-11 receptor activation attenuates lipopolysaccharide-induced systemic inflammation in mice, toxic nephritis, and leukocyte-mediated liver injury. Therefore, we propose that isoflurane-mediated induction of IL-11 and renal tubular IL-11 receptor activation produce powerful protection against ischemic AKI by targeting all three pathways of cell death: necrosis, apoptosis, and inflammation. Furthermore, as IL-11 as well as IL-11 receptors are expressed in many tissues and cell types, it remains to be determined whether isoflurane can induce IL-11 in nonrenal cells (e.g., hepatocytes, intestinal epithelial cells, and endothelial cells) and confer protection in these organs against AKI-induced remote organ injury.

The signal transduction mechanisms of IL-11–induced cytoprotection have been investigated in other cell types. IL-11 ligand and receptor complex interacts with a common receptor subunit, glycoprotein 130 (gp130), leading to gp130-associated kinase-mediated tyrosine phosphorylation. In cardiac myocytes, IL-11 reduces injury and fibrosis by the Janus kinase/signal transducers and activators of organs.

Fig. 7. Interleukin (IL)-11 is critical for isoflurane-mediated reduction in renal tubular necrosis after ischemia and reperfusion (IR). (A) Representative photomicrographs of five to six experiments for hematoxylin and eosin staining (magnification ×200) of kidneys of IL-11 receptor wild-type (IL-11R WT) mice, IL-11 receptor–deficient (IL-11R knockout [KO]) mice, and IL-11R WT mice pretreated with IL-11–neutralizing antibody and subjected to 30-min renal ischemia and 24-h reperfusion (I/R). Scale bars in all panels of A are 100 μm. (B) Summary of Jablonski scale renal injury scores (N = 4, graded from hematoxylin and eosin staining, scale 0–4) for mice subjected to renal I/R. *P < 0.05 versus pentobarbital-anesthetized IL-11R WT mice subjected to renal I/R. Error bars represent 1 SD. IL-11R WT mice anesthetized with pentobarbital after renal ischemia showed severe renal tubular necrosis. Isoflurane postconditioning significantly attenuated renal tubular necrosis and renal injury scores after renal IR. IL-11R deficiency (IL-11R KO) or IL-11 neutralization prevented renal protection with isoflurane postconditioning in mice.
transcription 3 activation.\(^3\)\(^8\),\(^4\)\(^3\) In vascular endothelial and intestinal epithelial cells, IL-11 protects against oxidant-induced necrosis and apoptosis via mechanisms involving extracellular signal-regulated kinase, mitogen-activated protein kinase, protein kinase B, and/or induction of heat shock protein 25.\(^3\)\(^7\),\(^4\)\(^5\),\(^4\)\(^6\)

Isoflurane-induced IL-11 generation may also produce antiinflammatory effects after renal I/R via modulating the nuclear factor-κB activity. Indeed, nuclear factor-κB is one of the most important proinflammatory transcription factors.\(^4\)\(^7\) IL-11 has been shown to attenuate transcription factor NF-κB in several cell lines and in mouse models of kidney inflammation.\(^4\)\(^0\),\(^4\)\(^8\),\(^4\)\(^9\) Furthermore, IL-11 treatment in vivo decreases glomerular nuclear factor-κB activity and reduces renal injury in experimental glomerulonephritis.\(^4\)\(^0\)

We previously showed that IL-11 induces renal protection by direct induction of sphingosine kinase-1 via nuclear translocation of hypoxia-inducible factor-1α.\(^1\)\(^6\) Sphingosine kinase-1 produces sphingosine 1-phosphate—a well-known antiinflammatory immune modulator.\(^5\)\(^1\) Based on our previous\(^9\),\(^5\)\(^2\) and current experimental data, we propose that isoflurane may directly induce several antiinflammatory signaling molecules (e.g., IL-11, sphingosine 1-phosphate) to protect against hyperinflammatory response after ischemic AKI. Interestingly, several of the IL-11–mediated cytoprotective signal transduction proteins (e.g., extracellular signal-regulated kinase, mitogen-activated protein kinase, protein kinase B, sphingosine kinase) are also activated with volatile halogenated anesthetics as we demonstrated previously.\(^1\)\(^2\),\(^1\)\(^3\),\(^1\)\(^7\)

We have shown here that isoflurane postconditioning produces potent renal protection against ischemic AKI via IL-11–mediated reduction in kidney tubule necrosis, apoptosis, and renal inflammation. We propose that after renal I/R injury, necrotic tubules cells release proinflammatory cytokines (e.g., monocyte chemotactic protein-1 release) that can further aggravate inflammation by stimulating resident kidney macrophages and dendritic cells.\(^4\) These cells in turn release additional proinflammatory cytokines (e.g., macrophage inflammatory protein 2α, keratinocyte-derived cytokine) to promote cytotoxic T-lymphocyte, neutrophil, and macrophage infiltration into the kidney interstitial space. Infiltrating proinflammatory leukocytes release additional cytotoxic proinflammatory and proapoptotic cytokines (e.g., tumor necrosis factor-α) which will exacerbate renal tubular cell necrosis as well as apoptosis. Figure 10 summarizes the potential mechanisms of isoflurane-mediated renal protection involving renal tubular IL-11 synthesis via TGF-β1 signaling.

Fig. 8. Interleukin (IL)-11 is critical for isoflurane postconditioning–mediated reduction in renal neutrophil infiltration after ischemia and reperfusion. (A) Representative photomicrographs of four to six experiments for immunohistochemistry (brown staining) for neutrophil infiltration (×200) from kidneys IL-11 receptor wild-type (IL-11R WT) mice, IL-11 receptor–deficient (IL-11R knockout [KO]) mice, and IL-11R WT mice pretreated with IL-11–neutralizing antibody and subjected to 30-min renal ischemia and 24-h reperfusion. Scale bars in all panels of A are 100 μm. (B) Quantifications of infiltrated neutrophils per ×200 field in the kidneys of mice after renal ischemia reperfusion (RIR). * \(P < 0.05\) versus vehicle-treated pentobarbital-anesthetized mice subjected to RIR. Error bars represent 1 SD. IL-11R WT mice anesthetized with pentobarbital after renal ischemia showed heavy neutrophil infiltration. Isoflurane postconditioning significantly attenuated renal tubular neutrophil infiltration after RIR. IL-11 deficiency (IL-11R KO) or IL-11 neutralization attenuated these reductions in renal neutrophil infiltration with isoflurane postconditioning in mice.
We believe that potential for clinical use of recombinant IL-11 therapy would be far superior to therapy against ischemic AKI with recombinant TGF-β1. Active form of TGF-β1 has a very short plasma half-life (2–3 min) as nonlatent TGF-β1 gets rapidly taken up by the liver, kidneys, lungs, and spleen and degraded. This is in contrast to the more prolonged half-life of recombinant IL-11 (approximately 7 h). Furthermore, prolonged exposure to TGF-β1 may promote kidney fibrosis. Therefore, prolonged TGF-β1 therapy may transiently reduce inflammation and necrosis but may cause increased renal tubular fibrosis that may paradoxically prolong renal dysfunction after I/R. Unlike TGF-β1, prolonged and high-dose IL-11 therapy does not induce tissue fibrosis.

We recently showed that isoflurane via TGF-β1 induces ecto-5′-nucleotidase (CD73) to generate cytoprotective adenosine in renal proximal tubule cells. CD73 induction and CD73-mediated adenosine generation were critical for isoflurane-mediated renal protection against ischemic AKI. However, the downstream target of isoflurane-mediated adenosine generation as well as the specific adenosine receptor subtype(s) responsible for renal protection against ischemic AKI remains unclear. We hypothesize that isoflurane-mediated induction of CD73 activity and adenosine generation directly stimulates renal tubular IL-11 synthesis to protect against ischemic AKI. Consistent with this hypothesis, we recently also showed that a specific A1 adenosine receptor agonist 2-chloro cyclopentyladenosine also induces IL-11 in proximal tubule cells. It remains to be tested in future studies whether isoflurane-mediated adenosine generation results in the activation of renal tubular A1 adenosine receptors to protect against renal ischemia and reperfusion injury.

There are several limitations to our study. Our results may not completely translate to clinical setting as differences in pathophysiology between human and mouse ischemic AKI exist. Furthermore, our studies focused on renal tubular synthesis of IL-11 and protection whereas clinical AKI results in both impairment of glomerular filtration and renal tubular dysfunction. In addition, our in vitro studies have limitation as HK-2 cells used in this study are immortalized and primary culture of proximal tubule cells may undergo rapid phenotypic changes ex vivo. Finally, complete isoflurane concentration–response curves were not generated in most of our experiments.

In summary, we demonstrated that a widely used volatile halogenated anesthetic isoflurane protects against renal
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Fig. 10. Proposed summary of cellular mechanisms of renal protection with postischemic isoflurane treatment. Collectively, our data suggest that anesthesia with isoflurane increases interleukin (IL)-11 messenger RNA and protein synthesis via transforming growth factor-β1 (TGF-β1) signaling. We propose that IL-11 synthesized subsequently activates interleukin-11 receptor (IL-11R) in neighboring renal tubules or endothelial cells to induce cytoprotective signaling. Because previous studies have shown that IL-11 reduces the activity of a well-known proinflammatory transcription factor NF-kB, it is highly possible that IL-11 generated with isoflurane treatment may also attenuate NF-kB activity to protect against renal inflammation and injury after acute kidney injury. SMADs are intracellular proteins that transduce extracellular signals from TGF-β1 to the nucleus to initiate downstream gene transcription. Hypothesis pathways (e.g., NF-kB inhibition) leading to cytoprotection are shown in dashed lines. We previously showed that IL-11 produces renal protection by direct induction of sphingosine kinase-1 via nuclear translocation of hypoxia-inducible factor (HIF)-1α. IR = ischemia reperfusion; NF-kB = nuclear factor-κ-light-chain-enhancer of activated B cells; PS = phosphatidylserine; SMAD = SMA (from Caenorhabditis elegans protein sma for small body size) and MAD (from Drosophila protein mothers against decapentaplegic) related family of transduction proteins.

References

tubular necrosis and inflammation after renal I/R by inducing cytoprotective IL-11 generation.

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References

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