Isoflurane protects against renal ischemia and reperfusion injury and modulates leukocyte infiltration in mice

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Lee HT, Kim M, Kim M, Kim NL, Billings FT, D’Agati VD, Emala CW, Sr. Isoflurane protects against renal ischemia and reperfusion injury and modulates leukocyte infiltration in mice. Am J Physiol Renal Physiol 293:F713–F722, 2007. First published June 27, 2007; doi:10.1152/ajprenal.00161.2007.—Inflammation after renal ischemia-reperfusion (IR) injury is a major contributor to renal cell death. We previously demonstrated that several volatile anesthetics protect against renal IR injury and necrosis in rats in vivo. We subsequently showed that volatile anesthetics produced direct anti-inflammatory and anti-necrotic effects in cultured proximal tubule cells in vitro. In this study, we wanted to determine whether the volatile anesthetic isoflurane protects against renal IR injury by producing anti-inflammatory effects in mice. C57BL/6 mice subjected to renal IR under isoflurane anesthesia demonstrated improved renal function and reduced necrosis compared with mice subjected to renal IR under pentobarbital anesthesia. Mice subjected to renal IR under isoflurane anesthesia also showed a reduction in inflammation evidenced by a reduced renal influx of neutrophils and macrophages, reduced ICAM-1 expression, less upregulation of proinflammatory mRNAs (TNF-α, ICAM-1, KC, and IL-1β) as well as reduced nuclear translocation of NF-κB 24 h after renal IR injury. Analysis of specific lymphocyte subset trafficking to the kidney using flow cytometry demonstrated that isoflurane anesthesia reduced intrarenal influx of CD3+, CD4+, CD8+, and NK1.1+ lymphocytes at 3 h after renal ischemia compared with pentobarbital anesthesia. However, only the differential reduction of NK1.1+ lymphocytes persisted 24 h after renal ischemia. Therefore, we conclude that isoflurane anesthesia significantly attenuated renal IR injury in mice by reducing inflammation and modulating leukocyte influx. In particular, neutrophils, macrophages, and NK1.1+ lymphocyte cell modulation may play a significant role in renal protection by isoflurane anesthesia.

acute renal failure; flow cytometry; immunohistochemistry; inflammation; volatile anesthetic

perioperative acute renal failure (ARF) is a frequent complication with no effective therapy (6, 11, 43). Development of perioperative ARF implies a poor prognosis and is frequently complicated by many other life-threatening conditions including respiratory failure, sepsis, and a multiorgan dysfunction syndrome. Unfortunately, the mortality and morbidity rate from perioperative ARF has changed little over the past 50 years (6, 11, 43).

We previously demonstrated that clinically utilized volatile anesthetics reduced necrosis and improved plasma creatinine after renal ischemia-reperfusion (IR) injury in rats (27). These protective effects were independent of the effects of volatile anesthetic on renal blood flow or blood pressure (27). Subsequently, we showed in cultured human renal proximal tubule (HK-2) cells that a volatile anesthetic sevoflurane reduced necrosis and inflammation via direct effects on ERK and Akt phosphorylation and HSP70 induction (26). Therefore, it appears that volatile anesthetics may reduce necrosis and inflammation by direct cellular protective effects on renal proximal tubules.

However, after in vivo renal IR injury, inflammatory changes that occur are complex and include systemic effects on multiple organs and cell types and thus are not limited to the renal proximal tubule cell (3–5, 38). Renal proximal tubule cells display a heightened inflammatory phenotype after IR injury and release proinflammatory cytokines/chemokines to attract proinflammatory leukocytes (neutrophils, macrophages, lymphocytes) into the kidney which further propagates the inflammatory responses in the kidney after renal IR injury. Indeed, evidence for the pathologic role of leukocytes in generating and perpetuating renal IR injury is accumulating. Modulation and reduction of infiltrating leukocytes, in particular neutrophils and macrophages, have been shown to reduce the degree of renal IR injury (15). In addition, lymphocytes, specifically T lymphocytes and NK1.1+ lymphocytes, may play an important role in generating renal injury after IR (1, 37, 40, 45). However, the trafficking of T and N lymphocytes into kidneys after renal ischemia has not been extensively studied. More importantly, the leukomodulatory effects of volatile anesthetics after renal IR injury have never been studied.

In this study of renal IR injury in mice, we wanted to determine whether a commonly used volatile anesthetic, isoflurane, 1) protected against renal injury, 2) reduced renal inflammatory changes, 3) modulated the renal influx of leukocytes (neutrophils, macrophages, lymphocytes), and 4) modulated renal lymphocyte subtype trafficking.

MATERIALS AND METHODS

In Vivo Renal IR Injury

All animal protocols were approved by the Institutional Animal Care and Use Committee of Columbia University (New York, NY). We subjected mice to renal IR injury with techniques previously described (25, 29). In brief, male C57BL/6 mice (25–30 g; Harlan Sprague Dawley, Indianapolis, IN) were anesthetized with intraperitoneal pentobarbital (50 mg/kg body wt, or to effect) or 1.2% of the volatile anesthetic isoflurane which equals 1× minimum alveolar concentration (MAC; defined as the concentration of the anesthetic at the

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alveolus that is needed to prevent movement in 50% of subjects in response to a painful stimulus). Pentobartital-anesthetized mice were allowed to breathe room air spontaneously, whereas isoflurane-anesthetized mice breathed spontaneously while receiving 1.2% isoflurane in room air as described below. Body temperature was monitored with a rectal probe and maintained at 37°C. In some mice, a carotid artery catheter was placed to measure systemic blood pressures. To initially anesthetize the mice with isoflurane, they were placed in airtight 2-liter chamber with inflow and outflow outlets (Braintree Scientific, Braintree, MA). The chamber temperature was maintained between 36 and 38°C. They were anesthetized initially to achieve immobility. One MAC isoflurane was delivered in room air at 5 l/min using an agent-specific Datex-Ohmeda vaporizer. The volatile anesthetic concentration was monitored by an infrared analyzer by sampling gas at the outflow hose. After achieving complete general anesthesia, the animals were removed from the chamber during anesthesia and allowed to breathe identical anesthetic concentrations through a nose cone connected in parallel to the gas chamber. Each animal was subjected to a midline laparotomy, right nephrectomy, and sham operation or 30 min of left renal ischemia during anesthesia. After 30 min of left renal ischemia, occlusion clips were removed, and the abdomen was closed in two layers. Each animal was returned to the chamber and allowed to breathe identical anesthetic concentrations spontaneously for an additional 3 h. Pentobarbitol-treated animals were returned to their cages to recover from anesthesia. The temperature was maintained between 36 and 38°C with frequent (every 10 min) measurements using an infrared temperature sensor (Quick-Temp, Linear Laboratories, Fremont, CA). In accordance with our animal care protocol, our animals (isoflurane or pentobarbital) receive a single injection of local anesthesia (1.25 mg/kg bupivacaine) instilled for analgesia subcutaneously following recovery from general anesthesia.

**Assessment of Renal Function After IR Injury**

Renal function was assessed by measurement of plasma creatinine 3 and 24 h after renal ischemia as described previously (25, 27, 28).

**Histological Examination to Detect Necrosis**

Morphological assessment on hematoxylin and eosin-stained kidney sections was performed by an experienced renal pathologist (VDD), who was unaware of the treatment each animal received. An established grading scale (scores of 0 to 4) for assessment of necrotic injury to the proximal tubules was used for the histopathological assessment of IR-induced damage, as outlined by Jablonski et al. (22).

**Assessment of Renal Inflammation**

*Types of assessments.* Renal inflammation after IR injury was determined with 1) measurements of renal cortical myeloperoxidase (MPO; a marker of leukocyte infiltration) activity, 2) measurements of neutrophil infiltration [with both hematoxylin and eosin staining and immunohistochemistry (IHC) analyses of neutrophils], 3) IHC for macrophage infiltration and intercellular adhesion molecule-1 (ICAM-1) expression, 4) measurements of mRNA encoding markers of inflammation, including TNF-α, IL-1β, ICAM-1, and keratinocyte chemoattractant (KC), 5) assessment of nuclear translocation of the proinflammatory transcription factor, NF-κB, and 6) immunophenotyping of T lymphocyte (CD3+, CD4+, CD8+) and NK1.1+ lymphocyte influx into the kidneys after IR with flow cytometry.

**Renal Cortical MPO Assay**

Twenty-four hours after renal ischemic injury, renal cortex (~200 mg) was dissected and MPO enzyme activity was detected as described (27).

**Histological Quantification of Neutrophil Infiltration**

An experienced pathologist (VDD) who was unaware of the treatment groups identified neutrophils on the basis of the localization of the cells and the morphological features of the nuclei of the cells in light microscopic assessments of hematoxylin and eosin-stained samples. Neutrophils were quantified in 75 randomly chosen microscopic fields (magnification ×400) in the corticomedullary junction, and results were expressed as neutrophils counted per square millimeter.

**Immunohistochemical Detection of Neutrophils, ICAM-1, and Macrophages**

We also immunohistochemically detected renal neutrophil infiltration. Fixed mouse kidney sections were deparaffinized in xylene and rehydrated through a graded ethanol series to water. After being blocked with 10% normal horse serum in PBS, the slides were stained for neutrophils, macrophage, or ICAM-1 in serial sections with rat anti-mouse primary antibodies (clone 7/4 for neutrophils, F4/80 for macrophages, CD54 for ICAM-1, Serotec, Raleigh, NC) for 30 min, horseradish peroxidase-conjugated rabbit anti-rat IgG (1/60 dilution) for 30 min, and diaminobenzidine reagent (Vector Laboratories, Burlingame, CA) for 10 min. Spleen was used as a positive control tissue.

**Isolation of Lymphocytes from Mouse Kidney and Flow Cytometry Analyses of Lymphocyte Subtypes**

Flow cytometric analysis was performed with a Quanta SC flow cytometer (Beckman Coulter, Miami, FL) to analyze the whole kidney leukocyte content. These studies assessed kidney content at 3 and 24 h of reperfusion of T cell (CD3, CD4, and CD8) and NK cells (PK136) in mice subjected to renal IR under pentobarbital or isoflurane anesthesia. In brief, kidney was extracted, minced, thoroughly crushed, and then passed through a 50-μm mesh prewetted with PBS containing 10% FBS. The kidney cell suspension was diluted with PBS containing 10% FBS and carefully layered on an equal volume of Histopaque-1083 (Sigma) to isolate mononuclear cells. Samples were centrifuged at 400 g for 30 min at room temperature. The lymphocyte interface was collected, washed twice with PBS with 10% FBS, and pelleted. The lymphocytes recovered from the mouse kidney (10⁶ cells in a volume of 100 μl) were preincubated with an anti-mouse CD16/32 (2.4G2) antibody for 10 min to block nonspecific antibody binding. Samples were then incubated with different combinations of antibodies (CD45+CD3+7AAD, CD3+CD4+7AAD, CD3+CD8+7AAD, or CD45+NK1.1+7AAD) for 20 min at room temperature. Flow cytometry was conducted under conditions to eliminate the interference of nonspecific staining by dead and nonleukocyte (e.g., renal) cells. For discrimination of viable and dead cells, samples were stained with 7-AAD for 5 min at room temperature. Anti-mouse CD45 was used to determine the total leukocyte cell number. Kidney lymphocyte infiltrations were expressed as the number of cells per kidney.

**Antibodies for FACS**

Purified rat IgG2a (clone YTH71.3) was purchased from Serotec (Oxford, UK). Anti-CD3e (clone 145–2C11) and anti-CD45 (clone 30-F11) were purchased from eBioscience (San Diego, CA). Anti-NK1.1 (LY-55; clone PK136), anti-CD4/3L3T4 (clone GK1.5), and anti-CD8α/Lyt-2 (clone 53-6.7) were obtained from Beckman Coulter (Fullerton, CA).

**Semiquantitative RT-PCR Assays of TNF-α, IL-1β, KC, and ICAM-1**

Three hours after renal ischemic injury, renal cortices including the corticomedullary junction were dissected and total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA). RT-PCR was performed with a Mastercycler EP gradient thermocycler (Eppendorf, Westbury, NY). Primers used for semiquantitative RT-PCR.

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performed to analyze the expression of the proinflammatory genes tumor necrosis factor-α (TNF-α), IL-1β, KC, and ICAM-1 as described previously (25, 26). Primers were designed to yield expected PCR products of 200 to 600 bp, to have a 50–60% GC content, and to all span an intron to distinguish products arising from genomic DNA contamination. The quantitative accuracy of our RT-PCR technique was first confirmed for each primer pair used. The PCR cycle number for each primer pair was optimized for linear increases in densitometric band intensity measurements with increasing PCR cycles from 14–22. The starting amount of RNA (0.25–1 μg) was also optimized for linear increase in densitometric band intensity measurements at an optimized cycle of PCR. The products were resolved on a 6% polyacrylamide gel and stained with Syber green (Roche, Indianapolis, IN), and the band intensities were quantified with a BioSpectrum automated imaging system attached to a personal computer (UVP Systems, Upland, CA).

Nuclear Protein Extraction and EMSA

Renal cortices including the corticomedullary junction were isolated, nuclear proteins were extracted, and EMSA was performed as previously described (26, 27).

Protein Determination

Protein content was determined with the Pierce Chemical (Rockford, IL) bicinchoninic acid protein assay reagent with BSA as a standard.

Statistical Analysis

The data were analyzed with Student’s t-test when comparing means between two groups. One-way ANOVA plus Tukey’s post hoc multiple comparison test was used when comparing multiple groups and one-way ANOVA plus Dunnett’s post hoc multiple comparison test was used when comparing multiple groups to a single control. The ordinal values of the Jablonski scale were analyzed by the Kruskal-Wallis nonparametric test with Dunn posttest comparison between groups. In all cases, a probability statistic <0.05 was taken to indicate significance. All data are expressed throughout the text as means ± 1 SE.

Materials

All drugs were in saline. Unless otherwise specified, all chemicals were obtained from Sigma (St. Louis, MO).

RESULTS

Isoflurane Anesthesia Improves Renal Function After Renal IR Injury in Mice

We determined in our preliminary studies that systemic arterial blood pressure and renal blood flow (Fig. 1) did not change significantly after induction of anesthesia with pentobarbital or 1.2% isoflurane. In addition, these hemodynamic parameters did not differ significantly between 1.2% isoflurane- and pentobarbital-anesthetized mice. The average systemic arterial pressures for the pentobarbital and 1.2% isoflurane group after induction of anesthesia were 135 ± 6 mmHg (n = 5) and 132 ± 6 mmHg (n = 4), respectively. Finally, we maintained the temperature (measured with an infrared temperature sensor during laparotomy and reperfusion under anesthesia) between 36 and 38°C at all times. Renal function of mice subjected to 30 min of renal ischemia and 1.2% isoflurane anesthesia. After 3 h of reperfusion, plasma creatinine rose significantly in both pentobarbital (Cr = 1.3 ± 0.1 mg/dl, n = 6)- or isoflurane-anesthetized mice (Cr = 0.9 ± 0.1 mg/dl, n = 6) compared with sham-operated mice at 3 h after surgery (Cr = 0.3 ± 0.02 mg/dl, n = 6) for pentobarbital-anesthetized, sham-operated mice and Cr = 0.3 ± 0.03 mg/dl, n = 5 for isoflurane-anesthetized, sham-operated mice). Plasma creatinine rose even higher at 24 h after renal ischemia in both pentobarbital (Cr = 2.5 ± 0.3 mg/dl, n = 11) or isoflurane-anesthetized mice (Cr = 1.3 ± 0.1 mg/dl, n = 6). However, isoflurane-anesthetized mice showed significantly less rise in plasma creatinine at both 3 h (P < 0.05) and 24 h (P < 0.001) after reperfusion (Fig. 2). Therefore, significant renal protection occurred with isoflurane anesthesia compared with pentobarbital anesthesia after renal IR injury.

Isoflurane Anesthesia Reduces Renal Necrosis in Mice After Renal IR

Compared with kidneys from sham-operated animals (Fig. 3A), 30 min of renal ischemia under pentobarbital anesthesia followed by 24 h of reperfusion resulted in significant renal injury demonstrated by severe tubular dilatation, tubular swelling and necrosis, medullary luminal congestion and hemorrhage, and the development of proteinaceous casts. Mice anesthetized with isoflurane during and after renal ischemia demonstrated markedly reduced histological features of necrotic renal injury after renal IR compared with mice anesthetized with pentobarbital (Fig. 3A). Quantitative assessment of renal tubular necrosis using the grading scores of Jablonski et al. (22) is shown in Fig. 3B. Histological grading at 24 h after 30 min of renal ischemia in pentobarbital-treated mice resulted in severe acute tubular necrosis (grade of 2.8 ± 0.4, n = 8). In contrast, mice anesthetized with isoflurane showed significantly improved renal morphology compared with pentobarbital-treated mice (grade of 0.8 ± 0.3, n = 5, P < 0.01).
Isoflurane Anesthesia Reduces Inflammation in Mice After Renal IR Injury

To determine whether isoflurane protected renal function, at least in part, by reducing inflammation in mice, we quantified renal inflammation in renal cortices after IR injury by five indexes: 1) MPO activity (marker of leukocyte infiltration), 2) quantification of neutrophil infiltration via manual counting of hematoxylin and eosin-stained slides as well as by IHC for neutrophils, 3) IHC for macrophage infiltration and ICAM-1 expression, 4) amount of mRNA encoding markers of inflammation (TNF-α, ICAM-1, IL-1β, and KC), and 5) measuring nuclear translocation of the proinflammatory transcription factor NF-κB.

Renal Cortical MPO Assay

Renal cortices isolated from C57 mice subjected to 24 h of reperfusion after 30 min of renal ischemia under pentobarbital anesthesia demonstrated increased MPO activity (3.5 ± 0.7 OD·min⁻¹·mg protein⁻¹, n = 4) compared with sham-operated control mice (1.5 ± 0.4 OD·min⁻¹·mg protein⁻¹, n = 4, P < 0.05). Mice anesthetized with isoflurane and subjected to renal IR had lower MPO activity (1.8 ± 0.7 OD·min⁻¹·mg protein⁻¹, P < 0.05, n = 4).

Immunohistochemical and Histological Detection of Neutrophils, Macrophages, and ICAM-1

Figure 4A demonstrates representative immunohistochemical detections of neutrophil infiltration into the corticomedullary junction in sham-operated mice or mice subjected to renal IR under either pentobarbital sodium or isoflurane anesthesia. Sham-operated C57 mice did not exhibit detectable levels of neutrophils infiltrating the corticomedullary junction of the kidney (n = 4). Mice subjected to IR injury under pentobarbital anesthesia showed an increase in neutrophil infiltration (45.5 ± 9.3 neutrophils/mm², n = 8) 24 h after IR injury. Isoflurane-anesthetized mice subjected to renal IR had a significantly reduced neutrophil influx into the kidney (13.5 ± 7.3 neutrophils/mm², n = 6).

Twenty-four hours after renal IR, ICAM-1 expression and macrophage infiltration increased greatly in mice anesthetized with pentobarbital. In contrast, mice anesthetized with isoflurane showed reduced ICAM-1 expression and macrophage infiltration (Fig. 4, B and C).
RT-PCR Assays of Proinflammatory Genes

Mice subjected to 30 min of renal IR injury under pentobarbital anesthesia demonstrated increased expression of mRNA encoding ICAM-1, TNF-α, KC, and IL-1β in renal cortices at 3 h after reperfusion. In contrast, the increase was attenuated in mice anesthetized with isoflurane anesthesia and subjected to renal IR (Fig. 5).

Nuclear Translocation of NF-κB After Renal IR Injury

Binding of NF-κB to the nuclear fractions isolated from the kidney cortices of mice that underwent sham operation, renal IR under pentobarbital anesthesia, or renal IR under isoflurane anesthesia are shown in Fig. 6A (representative of 5 separate experiments). IR injury increased the binding of NF-κB under pentobarbital anesthesia, whereas isoflurane anesthesia attenuated this increase in NF-κB binding (Fig. 6B).

FACS Analysis of Lymphocyte Subtypes

Utilizing flow cytometry, we identified subpopulations of lymphocytes that infiltrated the kidney following renal IR under pentobarbital or isoflurane anesthesia. Flow cytometry staining of kidney lymphocytes showed increased renal trafficking of CD3 (Fig. 7), CD4, CD8, and NK1.1+ lymphocytes in mice anesthetized with pentobarbital at 3 and 24 h after renal IR injury. Increases in these lymphocytes were more pronounced at 3 h after reperfusion compared with 24 h after reperfusion. Isoflurane-anesthetized mice were more protected from 3 h after reperfusion compared with 24 h after reperfusion. Isoflurane-anesthetized mice subjected to renal IR showed significantly less infiltration of CD3, CD8, and NK1.1+ lymphocytes (Fig. 8) at 3 h after IR. CD4 infiltration was not statistically different between pentobarbital and isoflurane group at 3 h after reperfusion. In contrast, 24 h after renal IR, only the reduction in NK1.1+ lymphocyte influx persisted in mice anesthetized with isoﬂurane. The CD4 and CD8+ T lymphocytes remaining in kidneys 24 h after IR in mice anesthetized with pentobarbital were similar in number to the T lymphocytes remaining in the kidneys of mice subjected to isoflurane anesthesia.

DISCUSSION

The major findings of this study are that isoflurane at a clinically relevant concentration (1 MAC) and duration (3 h) protected against renal IR injury in mice by improving renal function and reducing necrosis and inflammation. Moreover, significant reductions in neutrophil, macrophage, and lymphocyte renal influx were observed in mice subjected to renal IR under isoflurane compared with pentobarbital anesthesia. Immunophenotyping of lymphocyte subsets in kidneys after IR injury demonstrated that mice anesthetized with isoflurane had significantly less infiltration of CD3+, CD8+, and NK1.1+ lymphocytes at 3 h after renal ischemia compared with pentobarbital-anesthetized mice. However, only the reduction of NK1.1+ lymphocytes persisted 24 h after renal ischemia.

Inflammation after renal IR injury is a major contributor of renal cell death. Specifically, inflammation is an important mechanism to initiate and maintain renal cell injury as it potentiates both necrosis (30, 31) and apoptosis (12–14). Moreover, necrotic cells via the release of toxic intracellular contents can potentiate the inflammatory process further. Modulation of inflammation, therefore, may protect against renal failure after IR injury.

Volatile anesthetics are the most commonly used drugs during the perioperative period in the United States. Anesthesiologists routinely use inhalational anesthetics as the primary component of general anesthesia during the maintenance period of surgery. The anti-inflammatory properties of volatile anesthetics in nonrenal organs have been demonstrated previously. Isoflurane pretreatment reduced acute lung injury when
given 1 or 12 h before an endotoxin challenge (39). Continuous isoflurane anesthesia for 1 h after a lethal dose of *Escherichia coli* lipopolysaccharide resulted in a significant increase in survival of mice with a delayed inflammatory response (17). Isoflurane pretreatment for 30 min attenuated the decrease in blood pressure and endothelium-dependent vasodilation, the increase in systemic TNF-α, and the damage to the vascular endothelium associated with lipopolysaccharide-induced in-

Fig. 5. Densitometric quantifications of relative band intensities normalized to GAPDH from RT-PCR reactions of proinflammatory mRNA markers TNF-α, IL-1β, keratinocyte-derived chemokine (KC), and ICAM-1 from renal cortices (including outer medullary junction) of mice subjected to pentobarbital (PB) or isoflurane (ISO) anesthesia and sham operation (Sham) or renal IR injury for 3 h (n = 6 each). *P < 0.05 vs. appropriate sham. #P < 0.05 vs. PB IR group. Means ± SE.

Fig. 6. A: representative image of NF-κB EMSA of nuclear extracts from renal cortices (including outer medullary junction) from Sham or mice subjected to IR under PB IR or ISO IR. B: densitometric quantifications of relative band intensities from NF-κB EMSA (n = 5). *P < 0.05 vs. appropriate sham. #P < 0.05 vs. PB IR. Means ± SE.
flammation in rats (36). In addition, we demonstrated in rats that volatile anesthetics (including isoflurane) provided significant protection against renal IR injury with improved renal function, reduction in renal tubular necrosis, and improved preservation of renal proximal tubular architecture (27). However, the characterization of the anti-inflammatory effects of volatile anesthetics in the kidney in vivo has not been performed in previous studies.

Neutrophils, macrophages, and lymphocytes play important roles in initiating and propagating inflammation after renal IR (4, 5, 9, 10, 41). We demonstrate in this study that isoflurane anesthesia significantly reduced infiltration of leukocytes (neutrophils, lymphocyte subtypes, and macrophages) into the kidney after renal IR injury. The appearance of neutrophils and macrophages mediates antigen-independent mechanisms of tissue damage due to innate immunity activation (35). Renal neutrophil infiltration after IR injury is a well-known phenomenon and neutrophils participate in the pathogenesis of renal injury (19). Neutrophil infiltration begins early, within 4 h after the initiation of reperfusion and peaks at ~24 h after reperfusion. Reduction of renal neutrophil infiltration may have contributed to less renal injury after IR under isoflurane anesthesia by reducing the cellular damage from neutrophils releasing reactive oxygen species and several proteolytic enzymes (proteases, elastases, and MPO).

Macrophages also participate in renal injury after IR as they infiltrate the kidney in large numbers and generate proinflammatory cytokines. Day et al. (15) provided direct evidence that macrophages play a direct and important role in renal IR injury as depletion of macrophages with clodronate significantly protected mice against renal injury after IR. These findings were duplicated in rats recently (23). Our data also support this hypothesis as 24 h after renal IR injury large numbers of macrophages infiltrated the renal interstitium in mice anesthetized with pentobarbital. Isoflurane anesthesia reduced the severity of the macrophage infiltration and this reduction in macrophage infiltration may have contributed to the improved renal function with isoflurane anesthesia.

Although neutrophils and macrophages have been implicated in the pathogenesis of renal injury after IR during the early (neutrophils) and late (macrophages) reperfusion period, the roles for lymphocytes (T cells and NK cells) in renal injury have not been as rigorously studied. Lymphocyte infiltration is associated with antigen-dependent immune reactions and it is surprising to find that lymphocyte infiltration occurs early (within 3 h) after reperfusion. Burne et al. (8) demonstrated that CD4 cells are crucial in the pathogenesis of renal injury as CD4-deficient mice are protected against renal failure after IR injury. We utilized flow cytometry to perform immunophenotyping of lymphocyte subsets (CD3, CD4, CD8, and NK1.1+ cells) 3 and 24 h after renal IR. Specifically, we showed that intrarenal lymphocyte infiltration increased significantly at 3 h after reperfusion in mice subjected to isoflurane anesthesia compared with mice subjected to renal IR under pentobarbital anesthesia. This finding is significant as we observed less renal dysfunction in mice anesthetized with...
isoflurane compared with pentobarbital-anesthetized mice at 3 h after reperfusion. Anecdotal reports of immunomodulation, specifically plasma lymphopenia and reduced plasma cytokine levels after volatile anesthetic exposure, have been reported (2, 7, 32). Further studies are required to directly elucidate the cellular mechanisms of isoflurane-mediated reduction in lymphocyte influx into the kidney early after IR.

Interestingly, 24 h after IR, only the reduction in NK1.1+ lymphocytes persisted in isoflurane-anesthetized animals. Infiltrations of other T lymphocyte subtypes (CD3, CD4, CD8, and NK1.1) were reduced at 24 h compared with 3 h after IR and the quantity of CD3-, CD4-, and CD8-positive lymphocytes detected within the kidney was similar in pentobarbital- or isoflurane-anesthetized mice. In fact, the degree of lymphocyte infiltration was less than about twofold increased over the sham-operated mice anesthetized with pentobarbital or isoflurane at 24 h after renal IR. However, the protection against renal failure persisted in isoflurane-anesthetized mice. Perhaps, CD8 cells play an important role during the early phase of renal injury after IR. Moreover, it appears that the NK1.1+ cells play an important role in initiating and persistently maintaining renal injury after IR of the kidney. The pathophysiological role of NK cells in contributing to liver and cardiac IR injury has been described (24, 42). Further studies will be required to clarify the pathophysiological significance of these findings.

Our data are consistent with the previous studies showing relatively sparse detection of lymphocytes 24 h after renal IR injury (1). Our data are also consistent with the “hit and run” model of lymphocyte-mediated renal injury (16). Consistent with this “hit and run” hypothesis, lymphocyte content at 24 h after renal IR was significantly less compared with 3 h after renal IR. This hypothesis proposes that T lymphocytes may infiltrate the kidney during the early reperfusion period but rapidly exit the kidney resulting in reduced renal T lymphocyte counts at 24 h after IR. These T lymphocytes, however, leave behind multiple T cell-derived proinflammatory cytokines including IL-2 and IFN-γ to trigger and potentiate the inflammatory response after renal IR. In this study, isoflurane anesthesia resulted in a significant reduction of T lymphocytes at 3 h after renal IR and provided renal protection at 3 and 24 h after renal IR. We propose that isoflurane’s renal protection, at least in part, may be due to the modulation of early T lymphocyte infiltration after IR. Perhaps, isoflurane may attenuate the “hit” component of the “hit and run” hypothesis by attenuating the lymphocyte activation during the early reperfusion period.

With regard to the possible mechanisms of isoflurane-mediated modulation of early lymphocyte activation and infiltration, we previously demonstrated in cultured HK-2 cells that a volatile anesthetic sevoflurane directly reduced necrosis and inflammation via ERK and Akt phosphorylation and HSP70 induction (26). We also determined that volatile anesthetics at 1–2 MAC including sevoflurane and isoflurane reduced the inflammatory changes (NF-κB translocation and proinflammatory cytokine mRNA and protein expression after TNF-α treatment) in human lymphocyte (Jurkat cells), neutrophil (HL-60), and macrophage cultures (THP-1, unpublished observations, Lee HT et al.). Other investigators also demonstrated previously that several volatile anesthetics including isoflurane reduced the inflammatory changes as well as chemotaxis in cultured as well as freshly isolated leukocytes including lymphocytes, neutrophils, and macrophages (18, 20, 33, 34, 44). This ability of isoflurane to directly attenuate the inflammatory changes as well as chemotactic mobility of leukocytes may blunt leukocyte-mediated renal injury. We propose that isoflurane-mediated renal protection against IR injury is due to both the direct reduction in necrosis and inflammation of renal tubule cells as well as due to the reduction in inflammatory changes/chemotaxis in leukocytes associated with renal IR thus attenuating the “hit and run” response of renal IR injury mediated by the infiltration of lymphocytes.
It has been demonstrated recently that even a very short-term inhalation of isoflurane for induction of anesthesia reduces inflammatory plasma cytokine levels (21). Therefore, the immunomodulatory effects of anesthetics should be considered when interpreting data from experimental animal models of inflammatory diseases. The major limitations of this study are that the direct cellular mechanism(s) of isoflurane-mediated anti-inflammatory effects as well as the specific cell types involved in isoflurane-mediated renal protection are not elucidated.

In summary, our study demonstrates that isoflurane anesthesia provided powerful renal protection against renal IR injury in mice. Besides improvements in serum creatinine, isoflurane-treated mice showed significantly less necrosis and inflammatory responses such as IR injury, volatile anesthetics such as isoflurane selectively modulates NK1.1+/H11001 the reperfusion period while reducing early infiltration of kidney-infiltrating lymphocytes in renal ischemia-reperfusion injury. Inhibition of apoptosis induced by isoflurane-reperfusion prevents inflammation. J Clin Invest 104: 541–549, 1999.

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