Sevoflurane Protects against Acute Kidney Injury in a Small-Size Liver Transplantation Model

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Key Words

Liver transplantation • Sevoflurane • Acute kidney injury

Abstract

Background: Living donor liver transplantation (LDLT) patients run the risk of developing acute kidney injury (AKI) and subsequent chronic kidney disease, affecting morbidity and mortality. Sevoflurane has anti-inflammation properties, and renal ischemia/reperfusion under sevoflurane anesthesia resulted in drastic improvements in renal function. Extrahepatic metabolism of sevoflurane has been reported in patients undergoing liver transplantation, and might lead to nephrotoxicity. However, whether sevoflurane anesthesia is safe with regard to renal function in small-size liver transplantation needs further investigation. As neutrophil gelatinase-associated lipocalin (NGAL) is an early predictive biomarker of AKI, we looked at the renal effects of sevoflurane in a rat liver transplantation model using small-for-size grafts to investigate the changes of NGAL level and kidney histology. **Methods:** Sixty male Sprague-Dawley rats were randomly divided into 2 groups after 50% size liver transplantation. Rats were anesthetized with chloral hydrate or with sevoflurane and subjected to liver transplantation. Twelve rats in each group were used for the survival study and 6 rats

were used for the hemodynamic study. Six rats in each group were sacrificed 2 or 24 h after reperfusion. We harvested kidneys and serum for further analysis, including histological and functional parameters; TNF- α , IL-6 and NGAL immunoassay; expressions of myeloperoxidase (MPO) activity; and NF- κ B in renal tissues. **Results:** Rats in the sevoflurane group had significantly lower Scr 24 h after reperfusion compared with those in the chloral hydrate group. Rats in the sevoflurane group demonstrated significantly reduced NGAL concentrations compared with rats in the chloral hydrate group 2 h after reperfusion. Epithelial necrosis in the chloral hydrate group (3.2 ± 0.8) was greater than that in the sevoflurane group (1.5 ± 1.1 ; $p < 0.05$). Sevoflurane anesthesia resulted in significantly lower plasma TNF- α and IL-6 concentrations and reduced MPO concentrations 2 h after reperfusion ($p < 0.05$). NF- κ B protein levels 2 h after reperfusion increased by at least 110% in the chloral hydrate group relative to the sevoflurane group 2 h after reperfusion ($p < 0.05$). However, the urine inorganic fluoride concentrations increased significantly ($p < 0.001$) 2 h after reperfusion in the sevoflurane group ($6.1 \pm 1.5 \mu\text{mol} \cdot \text{l}^{-1}$) compared with the chloral hydrate group. **Conclusions:** Sevoflurane anesthesia can attenuate renal injury and modulate inflammatory cascades in small-size liver transplantation using rat models.

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In the past decades, the shortage of cadaver livers has triggered a rapid increase in living donor liver transplantation (LDLT) around the world. Multiorgan failure, including renal and hepatic failure, remains a leading cause of mortality and morbidity, affecting LDLT which is being proposed widely [1–3]. Factors during the transplant procedures implicated in the development of acute kidney injury (AKI) include acute changes in intraoperative hemodynamics resulting from suprarenal inferior vena cava occlusion, and pharmacologic agents influencing renal function, secondary to ischemia-reperfusion injury [3, 4]. Renal dysfunction after adult LDLT may occur due to persistent portal hypertension and a hyperdynamic state in patients with a small-for-size graft [5, 6]. The incidence of AKI in liver transplantation has been reported to range between 17 and 95% [3], and the rate in adult LDLT may be higher. This special patient population, for these reasons, poorly tolerates an additional insult to the kidney, which may have a detrimental impact on short- and long-term outcomes. Hence, early implementation of measures to preserve, halt or ameliorate the progression of renal dysfunction should be an integral part in the management of orthotopic liver transplant recipients, and every pharmacologic agent used during liver transplantation, such as anesthetics, should be fully considered.

Sevoflurane is one of the widely used volatile anesthetics during hepatobiliary surgeries, including LDLT, and its effects on hepatic blood flow, oxygen delivery and liver functions have been extensively evaluated [7]. Recently, anti-inflammation properties of sevoflurane have gained attention. Sevoflurane can modulate inflammatory cascades and ischemia/reperfusion (I/R) injury in many organ systems, including the kidney [8–10]. Lee [9] recently demonstrated that sevoflurane has protective effects against renal I/R injury in rats and produces antinecrotic and anti-inflammatory effects in *in vitro* cultures of proximal tubules [10]. Sevoflurane might be an alternative treatment to attenuate kidney graft injury by modulating inflammatory cascades and antinecrotic effects.

The effect of sevoflurane on renal function has been extensively investigated, and increasing evidence indicates that sevoflurane anesthesia does not impair renal function in healthy patients or patients with impaired renal function [11, 12]. However, whether sevoflurane affects renal tubular function in patients undergoing liver transplantation is not known, especially in patients un-

dergoing LDLT. The metabolism of sevoflurane produces inorganic fluoride ions, and the metabolic pathway in this process is found both in the kidney and liver [13–15]. Recently, Kharasch et al. [15] suggested that intrarenal production of fluoride ion might be a more important factor for nephrotoxicity than hepatic metabolism, which also causes increased plasma inorganic fluoride concentrations. During the anhepatic phase and immediately after reperfusion of the liver graft, there are transient alterations in the metabolic impairment in the liver, and the extrahepatic metabolism of sevoflurane has been observed [16, 17]. It can significantly increase urine inorganic fluoride levels, which is associated with increased NAG levels, a sensitive marker of proximal renal tubular injury [17]. LDLT is distinctly different from other liver transplantation methods because it is easier to suffer small-for-size graft injury from transient portal hypertension in the early phase after liver transplantation, and hepatic function is more difficult to recover [18]. Thus, extrahepatic metabolism of sevoflurane might be more significant, and more intrarenal F1 production might occur, which affects kidney function.

As neutrophil gelatinase-associated lipocalin (NGAL), an early predictive biomarker of AKI, has been successfully used in cardiac studies and transplantation studies, we looked at the renal effect of sevoflurane in a rat liver transplantation model using small-for-size grafts to investigate the changes of systemic hemodynamics, NGAL levels and kidney histology.

Methods and Materials

Animals

Male Sprague-Dawley rats, aged 8–10 weeks, weighing 200–250 g, purchased from the Animal Resource Center at Zhejiang University School of Medicine, were used as donors and recipients. All the animal research protocols used in this study were approved by the institutional laboratory review board and accorded with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1985).

Establishment of the Rat Nonarterialized Orthotopic Liver Transplantation Model

A rat model of nonarterialized orthotopic liver transplantation without venovenous bypass was used as described previously [19]. The lobe ligation technique was used to reduce the graft size on the backtable. The median lobe of the liver was selected to be the graft, and the median ratio of the graft weight-to-recipient liver weight (graft weight ratio) was 50.2% (range: 45–57). The graft was stored in cold saline with a target cold ischemic time of 80 min; 2 h or 24 h after reperfusion. Animals were sacrificed; and blood and kidney samples were collected for further analysis.

Experimental Design and Induction of Anesthesia

The experiment was conducted in 2 groups of rats: (1) chloral hydrate anesthetic plus liver transplantation, and (2) sevoflurane anesthetic plus liver transplantation. The rats were anesthetized with either intraperitoneal chloral hydrate ($200 \text{ mg} \cdot \text{kg}^{-1}$ body weight, or to effect) or with sevoflurane. They were allowed to breathe oxygen through a conical-shaped canine anesthesia mask and semiclosed circle absorption system on an electric heating pad under a warming light. Rats in the sevoflurane group were induced with 3–5% sevoflurane and maintained at 1.5–2.5% sevoflurane until 2 h postreperfusion in the recipients. Sevoflurane was carried in 100% oxygen and the flow of oxygen gas was set at $4 \text{ l} \cdot \text{min}^{-1}$ with the anesthesia apparatus. The sevoflurane concentration was adjusted according to the respiratory pattern and heart rate. Inspired and expired gas samples were collected from the respiratory circuit to analyze $P_{\text{ET}}\text{CO}_2$, oxygen and sevoflurane concentration. The same sevoflurane vaporizer was used for all the rats in this study. The rats were allowed to breathe spontaneously so as to avoid the potential effects of positive-pressure ventilation on cardiac output as well as on renal hemodynamics. We considered the possibility of effects of sevoflurane on ventilation and the subsequent impact of changes in PaCO_2 on our results.

Survival Study

Twelve rats in the chloral hydrate anesthetized-group and sevoflurane anesthetized-group were used for the survival study. Rats that lived for more than 7 days after transplantation were considered survivors.

Hemodynamic Study

Six rats in each group were used for the hemodynamic study. After induction of anesthesia, the left cervical arteries were cannulated by a catheter for measurement of mean arterial pressure (MAP). The catheter was connected via the pressure transducers (MLT1050 Blood Pressure, PowerLab System; AD Instruments Pty Ltd., Castle Hill, N.S.W., Australia). To compensate for insensible water loss and fasting period, each animal was given lactated Ringer's $6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ using a 2-channel infusion pump (ANNE; Abbott) during animal preparation and liver section.

Assessment of Renal and Liver Function after Reperfusion

Blood samples were collected from the recipients 2 and 24 h after reperfusion (6 rats for sampling at each time point) and processed within 2 h after collection. Blood collected in serum separator tubes was allowed to clot for 15–20 min and then centrifuged for 12 min at $1,000 \text{ g}$. Serum was collected and subsequently frozen at -20°C until further analysis: $50 \mu\text{l}$ for the measurement of serum creatinine activities, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reported in units per liter (Hitachi 747 Automatic Analyzer; Boehringer Mannheim GmbH, Mannheim, Germany); and $50 \mu\text{l}$ for NGAL immunoassay. Quantitative NGAL levels were measured with a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's instructions. Concentrated samples were diluted up to 80-fold with the manufacturer-provided diluent. Standards, samples and controls were run in duplicate, and the resulting chromogen was read at 450 nm with an additional 570-nm wavelength correction (Tecan, San Jose, Calif., USA). NGAL concentrations ($\text{ng} \cdot \text{ml}^{-1}$) were then calculated on the basis of the constructed standard curves on respective en-

zyme-linked immunosorbent assay plates. Standard curve R^2 values ranged from 0.9903 to 0.9987.

Renal Histology

Semiquantitative assessment of histologic damage was carried out as follows: kidney samples were cross-sectioned through their midportion, fixed in buffered formalin, and stained with hematoxylin and eosin. For each kidney, 2 representative cross sections stained with hematoxylin and eosin containing the cortex and medulla were examined for tubular changes with an Axiolab light microscope (Carl Zeiss, Hamburg, Germany). The examiner was blinded to the experimental conditions. All proximal and distal tubules were analyzed. The percentage of damaged tubules per section was used to measure histologic damage, typically appearing as tubular cell necrosis. The following light microscopic criteria for tubular cell necrosis were applied: nuclear pyknosis, karyorrhexis and/or disruption of cell membranes. The severity of tubular damage was graded on a scale from 1 to 5. The different grades were defined as follows: grade 0 = no damage, grade 1 (mild damage) = less than 10% tubular necrosis, grade 2 (mild-to-moderate damage) = 10–25% tubular cell necrosis, grade 3 (moderate-to-severe damage) = 25–50% tubular cell necrosis, grade 4 (severe damage) = 50–75%, and grade 5 (severe-to-very severe) = greater than 75% tubular cell necrosis.

TNF- α and IL-6 Immunoassay

Serum TNF- α and IL-6 levels were detected in a 96-well microtiter plate by using a commercial enzyme-linked immunosorbent assay kit (BioSource International Inc., Camarillo, Calif., USA) according to the manufacturer's guidelines. All samples were tested in duplicate. The plate was read on an ELx800 automated microplate reader (Bio-Tek Instruments Inc., Winooski, Vt., USA) at 450 nm. The concentrations of TNF- α and IL-6 were calculated from a standard curve and expressed in picograms per milliliters ($\text{pg} \cdot \text{ml}^{-1}$). The lower limit of detection for the enzyme-linked immunosorbent assay was $8\text{--}16 \text{ pg} \cdot \text{ml}^{-1}$.

Measurement of the Renal Myeloperoxidase Activity

Activity of myeloperoxidase (MPO), an enzyme stored in the azurophilic granules of neutrophils, was assayed using a spectrophotometric method to assess tissue neutrophil sequestration. 100 mg of frozen kidney was thawed and extracted for MPO after homogenization and sonication. The assay is based on the oxidation of 3,3', 5,5'-tetramethyl benzidine by MPO in the presence of H_2O_2 . MPO activity was calculated using a standard curve derived from a MPO standard (Calbiochem; EMD Bioscience, La Jolla, Calif., USA) and is expressed as $\text{mU} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ kidney.

Protein Isolation and Western Blotting for NF- κB

The animal tissues were lysed with ice-cold lysis buffer [62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v sodium dodecyl sulfate, 10% glycerol, 50 mM dithiothreitol and 0.01% w/v bromophenol blue or phenol red]. After sonication, the lysates were centrifuged and the protein concentrations were determined; furthermore, 20- μg proteins were separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred onto a polyvinylidene difluoride-plus membrane. The immunoblots were blocked with 5% milk, probed with a 1:100 dilution of the anti-phospho-p38 MAPK (Thr180/Thr182; all obtained from Cell Signaling Technology) overnight at 4°C and in-

Fig. 1. MAP was monitored throughout the experimental period. MAP at baseline was 80 ± 8.9 mm Hg. All animals exhibited a sharp drop in their MAP 5 min after portal vein clamping, and no significant differences in the R120 blood pressure were observed between the 2 groups. Results are expressed as averages $\pm$ SEM. BL = Baseline; C5 = 5 min after portal vein clamping; C15 = 15 min after portal vein clamping; R5 = 5 min after portal vein reperfusion; R15 = 15 min after portal vein reperfusion; R120 = 120 min after portal vein reperfusion; SEV = sevoflurane anesthesia; CH = chloral hydrate anesthesia.

cubated with the corresponding secondary antibodies for 1 h at room temperature. Quantitative analysis of the band density was performed using simultaneously blotted actin density to correct for protein content. The relative band intensities, expressed in arbitrary units of phospho-p38, in relation to the sham-operation group (6 rats underwent laparotomy without liver removal and implantation), were assessed by performing densitometry using a VersaDoc 5000 Imaging System (BioRad, Richmond, Calif., USA).

Concentrations of the Plasma and Urine Inorganic Fluoride

Blood samples obtained 2 and 24 h after reperfusion were collected in plastic heparinized tubes and immediately centrifuged. In each period of study, the procedure was as follows: empty the bladder immediately before clamping and collect the urine from the bladder catheter 2 h after reperfusion. We measured blood and urine inorganic fluoride concentrations. The liquid samples were naturally warmed to room temperature; an 0.5 ml aliquot was diluted to 10 ml with deionized water. Then total ionic strength adjustment buffer was added.

Blood Analysis

The PaCO_2 levels in heparinized arterial blood were measured using a Radiometer ABL 700 blood gas analyzer (Copenhagen, Denmark).

Statistical Analysis

All data are presented as means $\pm$ SD. Data between experimental groups were compared using a 2-tailed unpaired t test. $p < 0.05$ was recognized as statistically significant. All analyses were performed using SAS release 6.12 (SAS Institute, Cary, N.C., USA).

Results

Sevoflurane Anesthesia Improved 7-Day Graft Survival

The 7-day graft survival rate was significantly improved from 41.8% (5/12) in the chloral hydrate-anesthetized group to 75% (9/12) in the sevoflurane-anesthetized group ($p = 0.036$). Two rats in the chloral hydrate-anesthetized group died within 48 h.

Hemodynamics during Small-Size Liver Transplantation

In the experimental rat groups, MAP decreased slightly immediately after clamping the inferior vena cava and then increased gradually to a value of 50 mm Hg during the anhepatic stage. After a compensatory increase in the MAP during the initial reperfusion stage, the MAP increased gradually toward the value recorded at baseline after reperfusion in the experimental rats, regardless of the type of anesthesia method (fig. 1). Interestingly, no significant difference was observed among the 2 groups with regard to MAP 2 h after reperfusion, indicating that sevoflurane inhalation was not responsible for the observed MAP fluctuations in this rat model. The PaCO_2 levels were also compared and no differences were found between the 2 groups ($p > 0.05$).

The Effect of Sevoflurane on the Liver Function after Reperfusion

We examined the degree of liver dysfunction by measuring serum ALT and AST 2 and 24 h after reperfusion. There were no differences related to ALT and AST between the 2 groups after reperfusion ($p > 0.05$; fig. 2).

Sevoflurane Anesthesia Significantly Reduced Renal Injury after Reperfusion

We examined the degree of renal dysfunction by measuring plasma creatinine and the degree of renal tubular injury by measuring NGAL 2 and 24 h after reperfusion. Rats with sevoflurane anesthesia had significantly lower plasma creatinine at 24 h after reperfusion compared with rats with chloral hydrate anesthesia plus liver transplantation. There were no differences related to creatinine between the 2 groups 2 h after reperfusion. The trend of lower NGAL was obvious in the sevoflurane-anesthetized group after reperfusion, although no statistical difference was found at 24 h after reperfusion. Rats with sevoflurane anesthesia demonstrated significantly reduced NGAL concentrations compared with rats with chloral hydrate anesthesia 2 h after reperfusion ($p < 0.05$; fig. 2).

Fig. 2. Comparison of ALT and AST concentrations in plasma of rats anesthetized with sevoflurane (SEV) or chloral hydrate (CH) (a, b). Comparison of NGAL and creatinine concentrations in the SEV and CH groups (c, d). Results are expressed as averages $\pm$ SEM. * $p < 0.05$ vs. SEV group.

Fig. 3. Comparison of TNF- α , IL-6 concentrations in rats anesthetized with sevoflurane (SEV) or chloral hydrate (CH) (a, b). Comparison of activity of MPO in the renal of rats anesthetized with SEV or CH (c). Results are expressed as averages $\pm$ SEM. * $p < 0.05$ vs. SEV group.

Sevoflurane Anesthesia Significantly Attenuated TNF- α and IL-6 Concentrations after Reperfusion

Plasma TNF- α and IL-6 concentrations in rats with chloral hydrate anesthesia were significantly increased compared with the rats with sevoflurane anesthesia ($p < 0.05$; fig. 3). Sevoflurane anesthesia resulted in significantly lower plasma TNF- α and IL-6 concentrations 2 h after reperfusion ($p < 0.05$). Plasma TNF- α concentrations were also lower in the sevoflurane-anesthetized group compared with the chloral hydrate-anesthetized group 24 h after reperfusion ($p < 0.05$; fig. 3).

Renal MPO Activity 2 h after Reperfusion Is Reduced by Sevoflurane Anesthesia

MPO is an enzyme present in leukocytes and is an index of tissue leukocyte infiltration. Rats with sevoflurane anesthesia demonstrated significantly reduced MPO concentrations compared with rats with chloral hydrate anesthesia 2 h after reperfusion ($p < 0.05$; fig. 3).

Fig. 4. NF- κ B protein expression in renal whole cell lysate after reperfusion (2 and 24 h) (**a**, **c**). Densitometric values were normalized to actin and are expressed as percent of SHAM $\pm$ SD. * $p < 0.05$ compared with sham-operation group (SHAM). # $p < 0.05$ compared to chloral hydrate-anesthetized (CH) group (**b**, **d**). SEV group = sevoflurane-anesthetized group.

Protein Expression of NF- κ B in the Renal Cortical Is Reduced by Sevoflurane Anesthesia

NF- κ B protein levels 2 h after reperfusion increased by at least 180% in the chloral hydrate-anesthetized group relative to the sham operation group ($p < 0.05$), and increased by at least 110% in the chloral hydrate-anesthetized group relative to the sevoflurane-anesthetized group 2 h after reperfusion ($p < 0.05$). No differences were observed with regard to NF- κ B protein expression between the 2 groups 24 h after reperfusion (fig. 4).

Renal Histology

Epithelial necrosis in the chloral hydrate-anesthetized group (3.2 ± 0.8) was greater than that in the sevoflurane anesthetized group (1.5 ± 1.1 ; $p < 0.05$). Overall, proximal tubules appeared to be more susceptible to damage than distal tubules. The predominant location of epithelial necrosis in the chloral hydrate-anesthetized group was in the corticomedullary junction, as opposed to the sevoflurane-anesthetized group, which demonstrated necrosis in the medulla and cortex as well (fig. 5).

Plasma and Urine Inorganic Fluoride Concentrations Are Increased by Sevoflurane Anesthesia

The plasma inorganic fluoride concentrations 2 and 24 h after reperfusion in the sevoflurane-anesthetized group were 10.72 ± 1.30 and $4.73 \pm 2.31 \mu\text{mol} \cdot \text{l}^{-1}$, respectively. The urine inorganic fluoride concentrations increased significantly ($p < 0.001$) 2 h after reperfusion in the sevoflurane-anesthetized group ($6.1 \pm 1.5 \mu\text{mol} \cdot \text{l}^{-1}$) compared with the chloral hydrate-anesthetized group.

Discussion

LDLT patients run the risk of developing AKI and subsequent chronic kidney disease, affecting morbidity and mortality [3–6]. During orthotopic liver transplantation, at the point which reperfusion of the implanted liver is being performed, release of substance from the graft itself such as proinflammatory cytokines and oxygen free radicals occurs [20]. These substances can result in liver injury as well as renal injury. In addition to injury secondary to I/R, the venous warm ischemia from suprarenal inferior vena cava occlusion during transplantation is another confounding factor to AKI. Both renal venous and arterial I/R can result in renal injury, with venous renal outflow obstruction resulting in more severe functional renal injury compared to arterial inflow occlusion. Macrophage activation and neutrophilic infiltration appear to be exaggerated during venous occlusion [21]. Kidney injury to kidney can also be due to the unstable hemodynamics.

Lee et al. [9] demonstrated recently that volatile anesthetics reduced necrosis and inflammation in rats after renal I/R injury in vivo. Renal I/R under sevoflurane anesthesia resulted in drastic improvements in renal function, improved preservation of renal proximal tubular architecture, reduced necrosis and near-complete inhibition of neutrophil influx. However, no prior study has examined the renal effects of sevoflurane anesthesia in the small-size liver transplantation. To more accurately recapitulate the complex pathophysiology of human liver transplantation, we adopted 50% size liver implantation that produces a clinical picture which more accurately reflects I/R injury

Color version available online

Fig. 5. Representative light micrographs of rat kidneys. A hematoxylin-eosin stain of kidney sections from the sevoflurane-anesthetized (SEV) group 2 h after reperfusion (**a, c**), and from the chloral hydrate-anesthetized (CH) group 2 h after reperfusion (**b, d**). Magnification: $20\times$ (**a, b**); magnification: $40\times$ (**c, d**).

and small-for-size liver graft injury than animal models that use livers of other sizes. This model induces small-for-size liver graft injury and both renal venous and arterial I/R injury, and has been shown to resemble human liver transplantation with respect to cytokine generation and progression to organ failure. In the present study, we investigated the renal effect of sevoflurane using creatinine and NGAL, a potentially useful marker for the detection of early renal tubular injury in renal transplantation as well as liver transplantation patients [22–26].

Our study demonstrated that sevoflurane administration significantly reduced NGAL 2 h after reperfusion. To avoid confounding by systemic hemodynamics in this renal functional study, we applied 2–3% sevoflurane for induction and 1.5–2% for maintenance until 2 h after reperfusion in the recipients. Minimum alveolar concentration (MAC) of sevoflurane is reduced after portal vein clamping in our study. It was demonstrated that 1.0 MAC of sevoflurane given both during and after renal ischemia can protect against renal I/R injury in rats [9]. The reduc-

tion of anesthetic concentration was important to maintain an adequate level of anesthesia and minimize the negative impact of sevoflurane on cardiocirculatory parameters during the anhepatic phase and initial reperfusion phase [27, 28]. In our study, no significant difference was observed among the rat groups with regard to MAP during the entire operation and 2 h after reperfusion, indicating that this concentration of sevoflurane anesthesia failed to significantly reduce systemic blood pressure in rats. The trend of lower NGAL and creatinine was obvious in the sevoflurane anesthesia group after reperfusion. The administration of sevoflurane during transplantation and 2 h after reperfusion significantly attenuated renal injury. The significant lower plasma level of proinflammatory cytokines TNF- α and IL-6 were observed 2 h after reperfusion in the sevoflurane-anesthetized group. Therefore, the protection against renal injury by sevoflurane can probably be ascribed to the reduction of excessive inflammatory responses from I/R in the liver transplantation recipients. Furthermore, the reduction of MPO activity, which is re-

lated to tissue leukocyte infiltration, might also be important for ameliorating leukocyte activation and infiltration during liver transplantation. Complete occlusion of the inferior vena cava results in renal outflow obstruction. Experimentally, renal vein outflow obstruction has been shown to cause exaggerated neutrophilic infiltration and severe renal injury [21]. The intragraft protein expression of the proinflammatory cytokine induction NF- κ B was also remarkably inhibited in the group anesthetized with sevoflurane. In essence, the rats anesthetized with sevoflurane had significantly longer survival with good preservation of renal function and normal renal architecture compared with the rats anesthetized with chloral hydrate.

However, in addition to the anti-inflammatory and antinecrotic effects of sevoflurane, sevoflurane also demonstrated its nephrotoxicity function by means of increasing production of compound A and inorganic fluoride concentration in the urine and plasma [13, 15]. In this study, this nephrotoxicity function of increased production of compound A can be avoided by administration of sevoflurane at a total gas flow of $6\text{ l}\cdot\text{min}^{-1}$ using a semi-closed circle system with a soda-lime canister [17].

In addition to the liver, the main metabolic organ for the defluorination of sevoflurane, sevoflurane can also be metabolized renally in certain situations [15]. Van Obbergh et al. [16] and Kanbak et al. [17] demonstrated extrahepatic metabolism of sevoflurane both in children and adult undergoing liver transplantation. In the absence of liver microsomes, the kidney may be faced with metabolizing an excessive plasma level of sevoflurane and, thus, intrarenal production of fluoride may be elevated [17]. Van Obbergh et al. [16] observed that during the anhepatic phase of liver transplantation, inorganic fluoride levels increased in the study group receiving sevoflurane compared to the isoflurane group. Kanbak et al. [17] observed plasma inorganic fluoride concentrations increased in the neohepatic phase and reached a peak value at the first postoperative hour. The urine inorganic fluoride concentrations increased significantly during the anhepatic and neohepatic phases, and peaked at P₀₁. In our study, we did not measure the inorganic fluoride concentrations in urine during the anhepatic phase because of the insufficient urinary volume for analysis. Our data showed that both plasma and urine inorganic fluoride concentrations increased 2 h after reperfusion, and plasma inorganic fluoride concentrations also increased 24 h after reperfusion. With these findings, we hypothesized that intrarenal production of fluoride may be increased in the anhepatic phase. It is conceivable that these alterations may cause renal tubule injury. Some studies have suggest-

ed that an agent that impairs renal tubules causes elevated NAG excretion in a delayed fashion. Higuchi et al. [29] observed a peak on day 2 after exposure to inorganic fluoride. Shimada et al. [30] measured elevated levels after 12 h and a peak level after 48 h following exposure to mercury in rats. Since the sevoflurane mediated protection from renal I/R injury through anti-inflammatory and antinecrosis effects, it was not possible to estimate renal damage related to plasma and urine fluoride concentrations. In our study, the increased inorganic fluoride in the plasma and urine might be responsible for the slightly increased NGAL levels in the sevoflurane-anesthetized group 24 h after reperfusion. NGAL levels in the sevoflurane-anesthetized rats were lower than that in the chloral hydrate-anesthetized group at 2 h; however, there was no statistical difference at 24 h between the 2 groups. Administration of sevoflurane during small-size liver transplantation is safe concerning renal tubule function; the protective effects of sevoflurane against renal I/R injury might overwhelm the renal damage related to fluoride concentrations. Whether fluoride mediates sevoflurane's antinecrotic and anti-inflammatory pathways or directly reduces the renal protective effects by impairing the renal tubule remains to be elucidated.

A major strength of this study is that a widely used clinical anesthetic (sevoflurane) was shown to have a beneficial impact on survival and reduce plasma NGAL after reperfusion in small-size liver transplantation using rat models. A second major strength is that we used the rat nonarterialized orthotopic liver transplantation model, which closely resembles the pathophysiological changes seen during human liver transplantation. It can also be argued that this model is better as there were no confounding factors such as sepsis, multiorgan failure or exposure to nephrotoxins after the initial measurable period of ischemia. A limitation of this study is that we did not measure the urine fluoride concentrations because of the insufficient urinary volume for analysis during the anhepatic phase. An additional limitation could be the normal preoperative liver function of the rats we studied; it could be useful to study rats with abnormal preoperative liver function.

In conclusion, sevoflurane anesthesia performed using a semiclosed circuit system may be considered as a way to avoid nephrotoxicity in small-size liver transplantation rat model, and sevoflurane anesthesia can even attenuate renal injury by measuring creatinine and NGAL levels. However, further studies are needed to determine whether sevoflurane anesthesia is safe with regard to the renal function in LDLT for patients with abnormal preoperative liver function.

References

- Smith JO, Shiffman ML, Behnke M, Stravitz RT, et al: Incidence of prolonged length of stay after orthotopic liver transplantation and its influence on outcomes. *Liver Transpl* 2009;15:273–279.
- Davis CL: Kidney failure in liver transplantation: it is time for action. *Am J Transplant* 2006;6:2533–2534.
- Barri YM, Sanchez EQ, Jennings LW, Melton LB, et al: Acute kidney injury following liver transplantation: definition and outcome. *Liver Transpl* 2009;15:475–483.
- Cabezuelo JB, Ramirez P, Rios A, et al: Risk factors of acute renal failure after liver transplantation. *Kidney Int* 2006;69:1073–1080.
- Yamamoto S, Sato Y, Ichida T, et al: Acute renal failure during the early postoperative period in adult living-related donor liver transplantation. *Hepatogastroenterology* 2004;51:1815–1819.
- Lee SK, Park JB, Kim SJ, et al: Early postoperative renal dysfunction in the adult living donor liver transplantation. *Transplant Proc* 2007;39:1517–1519.
- Chibber A, Dziak J, Kolano J, Norton JR, Lustik S: Anesthesia care for adult live donor hepatectomy: our experiences with 100 cases. *Liver Transpl* 2007;13:537–542.
- Shayevitz JR, Rodriguez JL, Gilligan L, Johnson KJ, Tait AR: Volatile anesthetic modulation of lung injury and outcome in a murine model of multiple organ dysfunction syndrome. *Shock* 1995;4:61–67.
- Lee HT, Ota-Setlik A, Fu Y, Nasr SH, Emala CW: Differential protective effects of volatile anesthetics against renal ischemia-reperfusion injury in vivo. *Anesthesiology* 2004;101:1313–1324.
- Lee HT, Kim M, Jan M, Emala CW: Anti-inflammatory and anti-necrotic effects of the volatile anesthetic sevoflurane in kidney proximal tubule cells. *Am J Physiol Renal Physiol* 2006;291:F67–F78.
- Conzen PF, Kharasch ED, Czerner SF, Artru AA, Reichle FM, Michalowski P, et al: Low-flow sevoflurane compared with low-flow isoflurane anesthesia in patients with stable renal insufficiency. *Anesthesiology* 2002;97:578–584.
- Gentz BA, Malan TP Jr: Renal toxicity with sevoflurane: a storm in a teacup? *Drugs* 2001;61:2155–2162.
- Holaday DA, Smith FR: Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers. *Anesthesiology* 1981;54:100–106.
- Kharasch ED, Thummel KE: Identification of cytochrome P450 2E1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane and methoxyflurane. *Anesthesiology* 1993;79:795–807.
- Kharasch ED, Hankins DC, Thummel KE: Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. *Anesthesiology* 1995;82:689–699.
- Van Obbergh LJ, Verbeeck RK, Michel I, et al: Extrahepatic metabolism of sevoflurane in children undergoing orthotopic liver transplantation. *Anesthesiology* 2000;92:683–686.
- Kanbak M, Karagoz AH, Erdem N, et al: Renal safety and extrahepatic defluorination of sevoflurane in hepatic transplantations. *Transplant Proc* 2007;39:1544–1548.
- Man K, Lee TK, Liang TB, et al: FK 409 ameliorates small-for-size liver graft injury by attenuation of portal hypertension and down-regulation of Egr-1 pathway. *Ann Surg* 2004;240:159–168.
- Man K, Lo CM, Ng IO, Wong YC, Qin LF, Fan ST, Wong J: Liver transplantation in rats using small-for-size grafts: a study of hemodynamic and morphological changes. *Arch Surg* 2001;136:280–285.
- Bellamy MC, Galley HF, Webster NR: Changes in inflammatory mediators during orthotopic liver transplantation. *Br J Anaesth* 1997;79:338–341.
- Park Y, Hirose R, Dang K, Xu F, et al: Increased severity of renal ischemia reperfusion injury with venous clamping compared to arterial clamping in a rat model. *Surgery* 2008;143:243–251.
- Parikh CR, Devarajan P: New biomarkers of acute kidney injury. *Crit Care Med* 2008;36:S159–S165.
- Mori K, Nakao K: Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. *Kidney Int* 2007;71:967–970.
- Parikh CR, Jani A, Mishra J, Ma Q, Kelly C, Barasch J, et al: Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. *Am J Transplant* 2006;6:1639–1645.
- Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN, Lee HAT: Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. *Anesthesiology* 2006;105:485–491.
- Niemann CU, Walia A, Waldman J, Davio M, Roberts JB, Hirose R, Feiner J: Acute kidney injury during liver transplantation as determined by neutrophil gelatinase-associated lipocalin. *Liver Transpl* 2009;15:1852–1860.
- Mutoh T, Nishimura R, Kim HY, Matsunaga S, Sasaki N: Cardiopulmonary effects of sevoflurane, compared with halothane, enflurane, and isoflurane, in dogs. *Am J Vet Res* 1997;58:885–890.
- Heerdt PM, Pleimann BE: The dose-dependent effects of halothane on right ventricular contraction pattern and regional inotropy in swine. *Anesth Analg* 1996;82:1152–1158.
- Higuchi H, Sumikura H, Sumita S, et al: Renal function in patients with high serum fluoride concentrations after prolonged sevoflurane anesthesia. *Anesthesiology* 1995;83:443–445.
- Shimada H, Kiyozumi M, Kojima S: Effect of N-benzyl-D-glucamine dithiocarbamate on renal toxicity of inorganic mercury in rats. *Chem Pharm Bull* 1990;38:1429–1431.