Mechanisms of the beneficial effect of sevoflurane in liver ischemia/reperfusion injury[1]

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ABSTRACT

PURPOSE: To evaluate the underlying mechanisms by which sevoflurane protects the liver against ischemia/reperfusion injury evaluate the mechanism by which sevoflurane exerts this protective effect.

METHODS: Twenty-six rats were subjected to partial ischemia/reperfusion injury for 1h: one group received no treatment, one group received sevoflurane, and sham group of animals received laparotomy only. Four hours after reperfusion, levels of alanine and aspartate aminotransferases, tumor necrosis factor-α, and interleukins 6 and 10 were measured. Analyses of mitochondrial oxidation and phosphorylation, malondialdehyde content, histology, and pulmonary vascular permeability were performed.

RESULTS: Serum levels of alanine and aspartate aminotransferases were significantly lower in the sevoflurane group compared to untreated controls ($p<0.05$). The sevoflurane group also showed preservation of liver mitochondrial function compared to untreated controls ($p<0.05$). Sevoflurane administration did not alter increases in serum levels of tumor necrosis factor-α, and interleukins 6 and 10. Sevoflurane treatment significantly reduced the coagulative necrosis induced by ischemia/reperfusion ($p<0.05$). Pulmonary vascular permeability was preserved in the sevoflurane group compared to untreated controls.

CONCLUSION: Sevoflurane administration protects the liver against ischemia/reperfusion injury, via preservation of mitochondrial function, and also preserves lung vascular permeability.

Key words: Ischemia. Reperfusion. Liver. Lung. Cytokines. Rats.

Introduction

The reperfusion of tissue after a prolonged ischemia results in ischemia/reperfusion (I/R) injury, which can ultimately lead to multiple organ dysfunction in cases where a severe systemic inflammatory response occurs[1-3]. Ischemia of the liver, which can occur with hepatectomy, transplantation, trauma, or shock, deprives the organ of oxygen, leading to mitochondrial dysfunction and energy deficiency[4]. Swelling of hepatocytes and endothelial cells after reperfusion contributes to narrowing of sinusoidal blood vessels and platelet aggregation, thus occluding hepatic microcirculation. The subsequent inflammatory response produces reactive oxygen species and cytokines, causing further cell damage[5].

The liver can be protected from I/R injury to some extent with sevoflurane administration[6-9]. It is thought that sevoflurane attenuates the inflammation, as some volatile anesthetics have anti-inflammatory properties[10,11]. However, hepatic ATP levels have also been shown to decrease following I/R injury[4,12]. Thus, sevoflurane may also protect mitochondrial function by acting on ATP-dependent potassium channels[13,14]. The aim of the present study was to identify the underlying mechanisms by which sevoflurane protects the liver against I/R injury.

Methods

The experimental protocol was approved by the Ethics Committee for Animal Research from School of Medicine, Universidade de São Paulo (approval code 147/2012). Animals received care in accordance with the Guide for the Care and Use of Laboratory Animals.

Male Wistar rats weighing 230–280g were individually housed and kept under standard conditions (12-h light/dark cycle at 22–28°C) with free access to a standard rat chow and water.

Experimental design and study protocol

Animals were anesthetized with an i.p. injection of ketamine (30 mg/kg Ketalar; Cristalia, SP, Brazil) and xylazine (30 mg/kg Rompum; Bayer AG, Leverkusen, Germany) and subjected to orotracheal intubation and mechanical ventilation (Small Animal Ventilator model 683; Harvard Apparatus, Holliston, MA, United States). Body temperature was monitored throughout the procedure and maintained at 35–37°C. An upper median abdominal laparotomy was performed, and the pedicle to the left lateral and median hepatic lobes was dissected and occluded for 1h with a nontraumatic microvascular clamp. The abdominal incision was closed, followed by a reperfusion period of 4h. This model of partial hepatic ischemia induces ischemia in ~70% of the liver parenchyma without intestinal congestion[15].

A total of 39 rats were divided into three groups: sham (n = 13), with rats receiving a median laparotomy and liver manipulation without liver ischemia; no treatment I/R (NT) (n = 13); and sevoflurane treated I/R (Sevo) (n = 13). In sham and no-treatment groups, ketamine (15 mg/kg, i.p.) was administered as needed during the I/R period. In the sevoflurane group, sevoflurane (Abbot Laboratories, Chicago, IL, United States) was administered continuously via orotracheal tube through a calibrated vaporizer at a concentration of 2% expired fraction (1 MAC).

After the 4-h reperfusion, blood samples were collected via cardiac puncture, and animals were euthanized by cross-section of the abdominal aorta. Liver tissue was collected from ischemic segments for biochemical and histologic examinations. Pulmonary tissue was collected for vascular permeability analysis.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels

Serum AST and ALT levels were assayed using the optimized ultraviolet method (COBRAS MIRA; Roche Diagnostics, Basel, Switzerland). Results are expressed as U/L.

Oxidation and phosphorylation of liver mitochondria

Liver mitochondria were prepared as described previously[16]. Briefly, mitochondrial oxygen consumption was measured polarographically using an oxygraph (Gilson 5/6H; Gilson Inc., Middleton, WI, United States) in a closed reaction vessel fitted with a Clark oxygen electrode (YSI Inc., Yellow Springs, OH, United States) at 28°C. Mitochondrial activated (S3) and basal (S4) states were measured as the rate of oxygen consumption in the presence or absence, respectively, of ADP. S3 and S4 are reported as nmol oxygen per mg mitochondrial protein per min. The respiratory control ratio (RCR) was calculated as S3/S4 and the ADP/oxygen (ADP/O) ratio was calculated as moles of

ATP formed from ADP per atom of oxygen consumed as indices of mitochondrial oxidation and phosphorylation activities[17]. Mitochondrial protein content was determined by the method of Lowry *et al*.[18].

Lipid peroxidation analysis

Malondialdehyde (MDA) content was evaluated as a measure of lipid peroxidation. Ischemic liver tissues (100 mg/mL) were homogenized in 1.15% KCl buffer and centrifuged at 14,000 × *g* for 20 min. The supernatant was then added to a reaction mixture consisting of 1.5 mL 0.8% thiobarbituric acid, 200 µL 8.1% (v/v) sodium dodecyl sulfate, 1.5 mL 20% acetic acid (pH 3.5), and 600 µL distilled water and heated to 90°C for 45 min. After cooling to room temperature, the samples were cleared by centrifugation (10,000 × *g* for 10 min), and the absorbance was measured at 532 nm using malondialdehyde bis (dimethyl acetal) as an external standard. The content of lipid peroxides is expressed as nmol MDA per mg of protein[19].

Histologic analysis

Ischemic liver samples were fixed in 10% buffered formalin for standard hematoxylin and eosin staining. The same pathologist performed the histologic evaluations in a blinded manner. The severity of injury was scored according to the incidence of coagulative necrosis on a scale of 0 to 9: 0 = no necrosis, 3 = mild, 6 = moderate, 9 = severe (Table 1)[20].

TABLE 1 – Graduation of coagulative necrosis score.

Coagulative Necrosis	Score
No necrosis	0
Mild	3 (1X3)
Moderate	6 (2X3)
Severe	9 (3X3)

Determination of inflammatory mediators

Serum levels of tumor necrosis factor-α, interleukin-6, and interleukin-10 were determined by ELISA using commercially available kits (Invitrogen of Thermo Fisher Scientific, Waltham, MA, United States).

Lung tissue microvascular permeability analysis

Increases in lung microvascular permeability were quantified by the Evans blue dye (EBD) extravasation technique as described previously[21]. EBD (20 mg/kg of body weight) was injected via the dorsal penile vein 15 min before euthanasia. After blood sample collection, the lungs were perfused with 30–50 mL of 0.9% NaCl at 10 mL/min using a syringe pump (model 975; Harvard Apparatus) and weighed. One small fragment was dried at 60°C for calculation of total dry weight. To extract the dye, the lung was incubated with formamide (4 mL/mg of tissue) for 24h at room temperature, and the absorbance of the solution at 620 nm was measured on a microplate reader (ELX 808; Bio-Tek Instruments, Winooski, VT, United States). The results are expressed as µg of EBD per g of dry weight tissue to avoid undervaluation due to edema formation[22].

Statistical analysis

The data are expressed as mean ± SD or as median (interquartile range). Multiple group comparisons were assessed with analyses of variance or Kruskal–Wallis, followed by Tukey's tests. The R program version 2.15.2 (The R Foundation for Statistical Computing) was used for statistical analysis, and $p<0.05$ was considered statistically significant.

Results

Serum AST and ALT levels

Liver I/R resulted in significant increases in serum AST and ALT levels in no-treatment and sevoflurane groups compared to the sham group ($p<0.05$) (Figure 1). Treatment with sevoflurane significantly reduced AST and ALT levels compared to the no-treatment group ($p<0.05$).

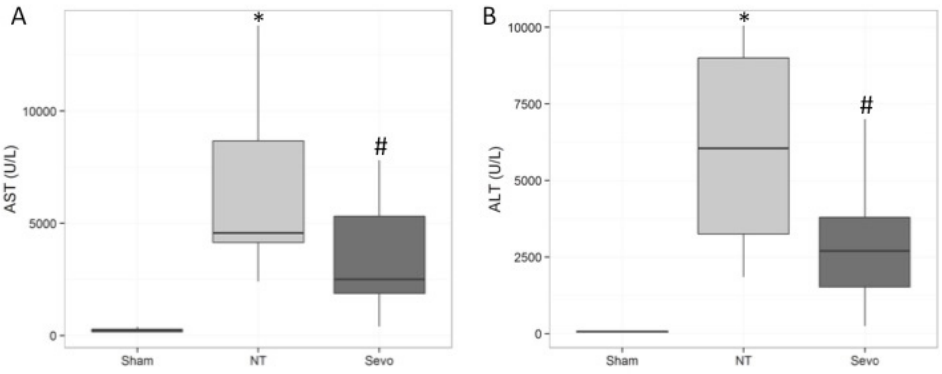

FIGURE 1 - Effects of sevoflurane on serum activities: (**A**) aspartate aminotransferase (AST) and (**B**) alanine aminotransferase (ALT) in liver/ischemia reperfusion (I/R). Sham group consisted of animals submitted to liver manipulation. Groups of animals submitted to liver I/R: No Treatment (NT): animals without sevoflurane administration during I/R injury time, and Sevoflurane group (Sevo): sevoflurane was administered during the whole I/R injury time. Data are expressed as median (quartile 25% - quartile 75%) *, # $p<0.05$

Oxidation and phosphorylation of liver mitochondria

After I/R, S3 and ADP/O values were significantly lower in the no-treatment group compared to sham and sevoflurane groups ($p<0.05$) (Figure 2). However, there was no difference in S3 values between the sham and sevoflurane groups. Compared to the sham group, RCR ratios were significantly decreased in the no-treatment and sevoflurane groups ($p<0.05$), however the sevoflurane group showed a significant increase when compared to the no-treatment group ($p<0.05$). No differences in S4 levels were found among the three groups.

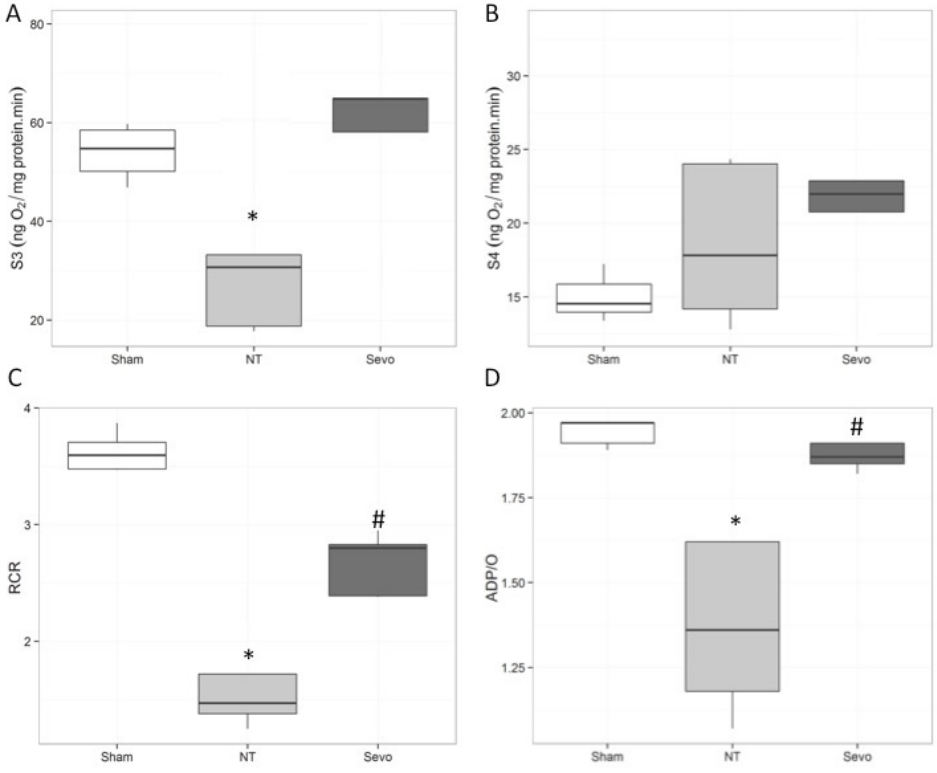

FIGURE 2 - Effects of sevoflurane on liver mitochondrial oxidation and phosphorylation activities in liver/ischemia reperfusion (I/R). (**A**): State 3 respiration (S3), (**B**): State 4 respiration (S4), (**C**): Respiratory control rate (RCR), and (**D**): ADP/O ratio. Sham group consisted of animals submitted to liver manipulation. Groups of animals submitted to I/R: No Treatment (NT): animals without sevoflurane administration during I/R injury time, and Sevoflurane group (Sevo): sevoflurane was administered during the whole I/R injury time. Data are expressed as median (quartile 25% - quartile 75%) *, # $p<0.05$

Liver MDA content

There were no differences in ischemic liver MDA content among groups (data not shown).

Liver histology

I/R induced necrosis in liver samples, observed as a significant increase in the coagulative necrosis score in the no-treatment and sevoflurane groups compared to the sham group ($p<0.05$) (Figure 3). However, sevoflurane administration significantly reduced the severity of necrosis when compared to the untreated group ($p<0.05$).

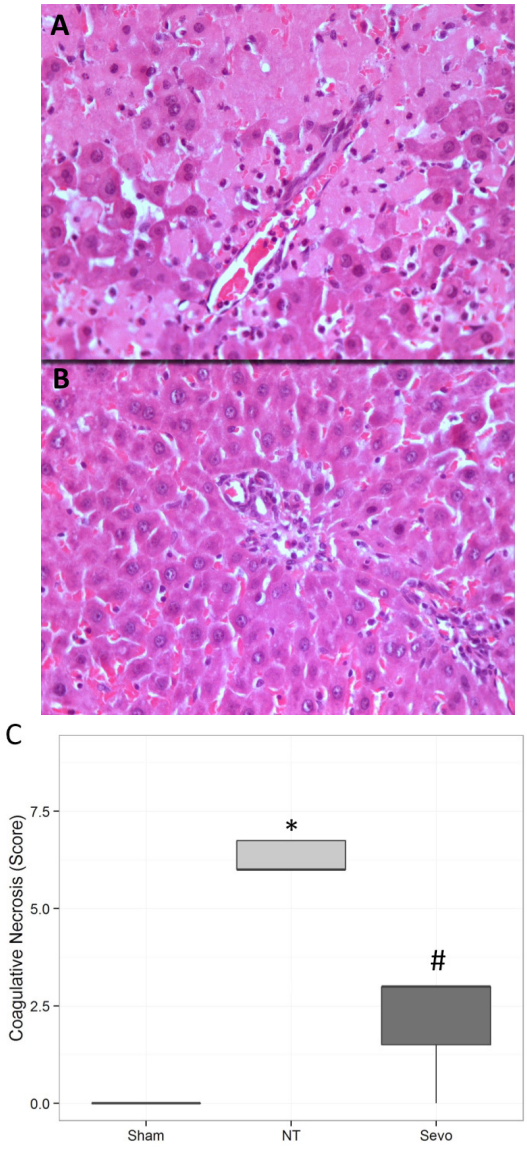

FIGURE 3 - Liver coagulative necrosis. (**A**) No-treatment group and (**B**) Sevoflurane group (hematoxylin and eosin; x400). (**C**) Quantification of necrosis. NT, no-treatment; Sevo, sevoflurane treated. Data are expressed as median (interquartile range); *p<0.05 *vs* sham; #p<0.05 vs NT.

Serum levels of inflammatory mediators

I/R resulted in a significant increase in serum levels of tumor necrosis factor-α, interluekin-6, and interleukin-10 in the no-treatment and sevoflurane groups compared to the sham group ($p<0.05$) (Table 2). However, levels were not different between the sevoflurane and no-treatment groups.

TABLE 2 - Serum levels of TNF-α, IL-6, and IL-10.

	TNF-α (pg/ml)	IL-6(pg/ml)	IL-10(pg/ml)
Sham	3 ± 5*	16 ± 27*	18 ± 19*
No Treatment	105 ± 62	684± 264	240 ± 105
Sevoflurane	66 ± 47	779 ± 421	296 ± 48

Data are presented as mean ± SEM
*p<0.05 *vs* any group

Lung microvascular permeability

I/R significantly increased lung microvascular permeability as assessed by EBD extravasation ($p<0.05$) (Figure 4). Sevoflurane treatment completely abrogated this, with no difference between the sham and sevoflurane groups.

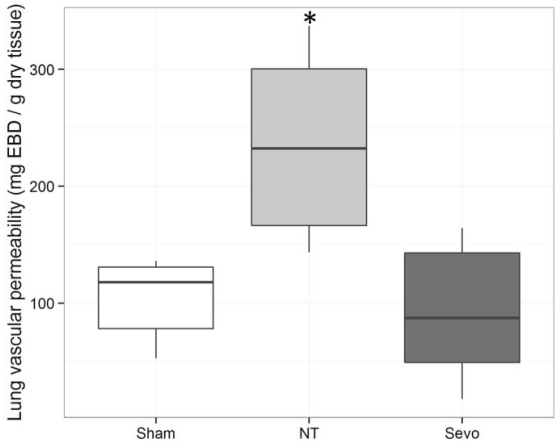

FIGURE 4 - Effects of sevoflurane on lung permeability analysis evaluated through Evans blue dye (EBD) extravasation in liver/ischemia reperfusion (I/R). Sham group consisted of animals submitted to liver manipulation. Groups of animals submitted to liver I/R: No Treatment (NT): animals without sevoflurane administration during I/R injury time, and Sevoflurane group (Sevo): sevoflurane was administered during the whole I/R injury time. Data are expressed as median (quartile 25% - quartile 75%) *p<0.05

Discussion

Hepatic I/R injury alters liver mitochondrial function, resulting in degeneration and necrosis in the liver that is

characteristic of cellular ischemia. The findings presented in this study show that sevoflurane restores mitochondrial function as a mechanism to protect the liver against I/R injury. This was observed as preserved S3 state respiration, RCR, and ADP/O ratios, as well as by reduced coagulative necrosis as assessed by histology. The preservation of mitochondrial function by sevoflurane resulted in reduced hepatic parenchymal damage and lower transaminase levels. It is possible that sevoflurane activated mitoK_{ATP} channels[22-27] and therefore decreased the intracellular calcium concentration, thus reducing mitochondrial damage in hepatic I/R injury.

Although previous studies have indicated that sevoflurane suppresses the production of reactive oxygen species and reduces lipid peroxidation in liver parenchyma[6,7], the results presented here failed to show significant differences in liver MDA content among the groups. Additionally, sevoflurane did not significantly reduce serum levels of inflammatory factors, which is consistent with previous studies[6,7]. This suggests that the protection afforded by sevoflurane administration is independent of the release of proinflammatory cytokines from activated Kupffer cells in I/R injury[28,29].

Analyses of EBD extravasation revealed that sevoflurane preserves lung microvascular permeability. As no reduction in inflammatory mediators was observed, we speculate that sevoflurane has a local beneficial effect in the lungs. This hypothesis is supported by recent evidence showing a protective effect of sevoflurane during lung ventilation thoracic surgery[28] and reduction of lung I/R injury[29].

Conclusion

The mechanism by which this occurs cannot be fully addressed by this study alone, but it probably involves preservation of mitochondrial function and protection of lung parenchyma, without a reduction of the systemic inflammatory response.

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