Sevoflurane exerts brain-protective effects against sepsis-associated encephalopathy and memory impairment through caspase 3/9 and Bax/Bcl signaling pathway in a rat model of sepsis

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Abstract

Objective: We compared the effects of sevoflurane and isoflurane on systemic inflammation, sepsis-associated encephalopathy, and memory impairment in a rat sepsis model of cecal ligation and puncture (CLP)-induced polymicrobial peritonitis.

Methods: Twenty-four rats were assigned to sham, CLP, CLP + sevoflurane, and CLP + isoflurane groups. At 72 hours after CLP, the rats underwent behavior tests. Serum cytokines were evaluated. Brain tissue samples were collected for determination of glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase; the wet/dry weight ratio; myeloperoxidase (MPO) and malondialdehyde (MDA); apoptotic gene release; and histologic examinations.

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Results: The MPO level, wet/dry weight ratio, and histopathology scores were lower and the Bcl2a1 and Bcl2l2 expressions were upregulated in both the CLP + sevoflurane and CLP + isoflurane groups compared with the CLP group. The interleukin-6, interleukin-1β, MDA, and caspase 3, 8, and 9 levels were lower; the GPX, SOD, Bax, Bcl2, and Bclx levels were higher; and non-associative and aversive memory were improved in the CLP + sevoflurane group compared with the CLP + isoflurane group.

Conclusion: Sevoflurane decreased apoptosis and oxidative injury and improved memory in this experimental rat model of CLP. Sevoflurane sedation may protect against brain injury and memory impairment in septic patients.

Introduction

Sepsis-associated encephalopathy (SAE) is defined as global brain dysfunction that occurs during sepsis without the presence of central nervous system infection, intracranial defects, or hepatic or renal dysfunction.[1] The reported incidence of SAE may reach 71%, and SAE may result in cognitive dysfunction, delirium, or deep coma.[2] Mortality due to sepsis is higher in patients with SAE than in patients without brain involvement, and survivors often experience neurological and psychological morbidities and memory impairment.[3]

Although the pathophysiology of SAE is not well established, studies have suggested that systemic inflammation, loss of blood–brain barrier integrity due to oxidative damage, and apoptosis in the brain are the main mechanisms resulting in SAE.[1,3–5]

The systemic inflammatory response induces secretion of cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6).[6–8] Myeloperoxidase (MPO) is a peroxidase enzyme abundant in neutrophil granulocytes, and the MPO level in tissue is an indicator of neutrophil migration reflecting inflammation.[9] In sepsis, oxidative stress and reactive oxygen species cause excessive lipid peroxidation, which results in organ dysfunction.[10] Malondialdehyde (MDA) is produced by reactive oxygen species-induced fatty acid peroxidation and has been shown to induce apoptosis.[11]

In addition to inflammatory toxicity and oxidative stress, neuronal apoptosis contributes to sepsis-related brain injury.[12] It is conceivable that apoptosis of neurons leads to SAE because its association with cognitive dysfunction in patients with sepsis has been well documented.[13,14] Studies have shown that agents that control the apoptotic pathways and reduce the number of apoptotic neurons might provide a therapeutic approach against SAE.[15,16]

Recent clinical studies have suggested sedation of patients with volatile agents in the intensive care unit.[17,18] Various inflammation models have demonstrated the beneficial immunomodulatory effects of volatile anesthetic agents.[19,20] Studies have shown that neuroprotection with volatile anesthetics may be mediated by suppression

of apoptotic pathways.[21,22] Nevertheless, the underlying mechanisms of their neuroprotective effect is complex, and the effects of sevoflurane and isoflurane on the antioxidant capacity and expression of genes involved in apoptosis have not been clearly defined.

This study was designed to compare the effects of sevoflurane and isoflurane on systemic inflammation, SAE, and memory impairment in a male rat sepsis model of polymicrobial peritonitis caused by cecal ligation and puncture (CLP). We hypothesized that lipid peroxidation, antioxidant capacity, and apoptosis-related gene expression in the brain might be regulated by sevoflurane and isoflurane inhalation, providing protection against memory-learning deficits.

Materials and methods

The Animal Research Committee at Gazi University, Ankara (GUET 10.020) approved this study. All animals were maintained in accordance with the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. This study was performed according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Twenty-four male Wistar rats weighing 375 to 425 g and aged 10 weeks were enrolled in this study. The animals were obtained from the Laboratory of Experimental Animals, Gazi University and housed at 21°C to 23°C with 30% to 40% humidity and a 12-hour light/dark cycle with *ad libitum* access to rat chow and water. Anesthesia was induced and maintained in all rats by intramuscular injection of 50 mg/kg of ketamine hydrochloride, 5 mg/kg of xylazine, and an intravenous fentanyl infusion at 2 μg/kg/hour. The depth of anesthesia was determined by the toe pinch–response method.

The rats were intubated and mechanically ventilated at a tidal volume of 6.5 mL/kg and respiratory rate of 100/minute (Inspira Animal Ventilator; Harvard Apparatus, Holliston, MA, USA). Femoral arterial and venous catheters were applied to monitor the mean arterial pressure (MAP), permit blood sampling for measurement of blood gas tensions, and maintain fluid infusion. Hydration was maintained by infusion of isotonic sodium chloride solution at 0.5 mL/hour. Sepsis was induced by establishing a CLP model as previously described.[4] Briefly, a midline incision was made in the lower abdomen; the cecum was then identified, ligated, punctured, and gently squeezed to ensure that feces were extruded. The intestinal tract was returned to the abdominal cavity, and the abdomen was completely closed.

The rats were randomly preassigned to one of four groups according to a computer-generated list: the sham (n = 6), CLP (n = 6), CLP + sevoflurane (n = 6), and CLP + isoflurane (n = 6) groups. Rats in the CLP, CLP + sevoflurane, and CLP + isoflurane groups underwent CLP for the initiation of sepsis. In the sham group, the abdominal cavity was exposed without CLP by a midline incision. After the CLP surgery and sham procedure, the rats in the CLP + sevoflurane group received 2% sevoflurane in 50% oxygen/air, the rats in the CLP + isoflurane group received 1.4% isoflurane in 50% oxygen/air, and the rats in the sham and CLP groups received only 50% oxygen/air via a calibrated flowmeter and vaporizer (Datex-Ohmeda, Madison, WI, USA) for 90 minutes. The rats were then extubated and returned to their cages. All rats received ceftriaxone at 30 mg/kg and clindamycin at 25 mg/kg every 6 hours for 72 hours. Arterial blood samples for blood gas analyses were obtained at baseline and at 24, 48, and 72 hours after CLP. At 72 hours, the animals underwent behavior tests. Blood was then

withdrawn, the rats were decapitated, and brain samples were collected.

Brain tissue fixation was performed as described by Gage et al.[23] Briefly, after the rats were anesthetized, the thorax was opened and a cannula inserted into the left ventricle. The rats were then transcardially perfused with 200 mL of ice-cold 0.1 M phosphate-buffered saline followed by 150 mL of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were dissected, put on ice, and separated to the right and left hemispheres. The right hemisphere was snap-frozen in liquid nitrogen and stored at -80°C for further analysis, and the left hemisphere was immersed in 4% paraformaldehyde overnight and excised for histological examination.

Cytokine measurement

The blood samples were centrifuged, and the plasma was separated and stored at -80°C. The plasma TNF-α, IL-6, and IL-1β levels were measured in duplicate with an enzyme-linked immunosorbent assay kit (Jingmei Biotech, Beijing, China) according to the manufacturer's guidelines. The results are expressed as pg/mL.

Measurement of cerebral edema

The brain water content was measured by the wet/dry weight method to determine the presence of cerebral edema.[24] After measurement of the wet weight, the brain tissues were dried for 24 hours to obtain the dry weight. The tissue water content is expressed as the percentage of wet tissue weight as follows: water content % = (wet weight – dry weight) / wet weight $\times$ 100%.

Measurement of brain superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX)

Brain tissue samples were sonicated in phosphate buffer and centrifuged to obtain cell supernatant. The cell supernatants were used for enzyme assay evaluations of GPX, SOD, and CAT activities. Absorbance readings were monitored at 240 nm and 25°C for 2 min using an ultraviolet-visible recording spectrophotometer (UV-160; Shimadzu, Kyoto, Japan) with a reference containing the working solution. The protein contents of the supernatants were measured using the Bradford method and are expressed as U/g.

Brain tissue MDA activity assay

The brain tissue samples were homogenized in ice-cold 1.15% KCl buffer, and the obtained homogenate was added to a mixture containing of 1.5 mL of 0.8% thiobarbituric acid, 200 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid (pH 3.5), and 600 mL of distilled water. The samples were centrifuged, and absorbance was measured at 532 nm. The lipid peroxide levels were then measured using the Bradford method and are expressed nmol MDA/mg protein.

Brain tissue MPO activity assay

The brain tissue samples were homogenized in 0.5% hexadecyltrimethylammonium bromide in 10 mM 3-N-morpholinopropanesulfonic acid and centrifuged at $15,000 \times g$ for 40 minutes to obtain a suspension. The suspension was sonicated, and the obtained supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM hydrogen peroxide. MPO activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37°C by a SpectraMax microplate reader (Molecular Devices, San Jose, CA, USA) using the Bradford method. The results are expressed as nmol MPO/mg protein.

RNA isolation. Brain tissues were homogenized using a Tri-reagent protocol.

After isopropanol precipitation, DNA-free total RNA samples were further purified using a genomic DNA-free total RNA isolation kit (Qiagen Sciences Inc., Germantown, MD, USA). Cells were lysed by the lysis solution provided in the kit and treated with RNase-free DNAase as instructed before passing through RNA isolation columns. The extracted RNA was quantified and stored at $-80°C$ until use.

A reverse transcription (RT) polymerase chain reaction (PCR) kit (RT Profiler PCR Array kit; SuperArray Bioscience Corporation, Frederick, MD, USA) was used to assess gene expression. The rat Apoptosis RT Profiler PCR Array was used to analyze the expression of 84 apoptotic genes, including TNF ligands and their receptors, Bcl2 family members, caspases, inhibitor of apoptosis, caspase recruitment domain family members, death domain, death effector domain, and cell death-inducing DFFA-like effector family members as well as the genes involved in p53 and DNA damage-induced apoptosis pathways. For each sample, 1 μg of RNA was converted into cDNA by an RT reaction. A LightCycler 480 (Roche Diagnostics, Indianapolis, IN, USA) was used to perform real-time PCR. The results were calculated using the fold-increase values of gene expression according to the manufacturer's instructions. A ≥2-fold increase or ≥2-fold decrease in the expression of a particular gene over control values was considered a statistically significant ($P < 0.05$) change.

Open field test. The open field test was performed to evaluate motor performance in the training section and non-associative memory in the retention test session. A square plastic box of 60×60 cm with 50-cm-high walls was used as the open field. The ground floor of the open field was divided into nine equal squares by black lines. For the training session, the animals were gently placed in the left rear quadrant of the apparatus and allowed to explore for 5 minutes. Immediately after the test session, the animals were returned to their cages. Twenty-four hours later, the animals underwent another test session in the same open field. Open field movements were recorded by a TRU SCAN 2.0 system (Coulbourn Instruments, Whitehall, PA). The numbers of times that the animals crossed the black lines and reared in both the training and test sessions were counted. A decrease in the number of crossings and rearings between the two sessions was used as the measure of retention of habitation.[25]

Step-down inhibitory avoidance test. This test was performed to evaluate aversive memory. The test was carried out in a 50- $\times$ 25- $\times$ 25-cm box containing parallel stainless steel bars spaced 1 cm apart in the floor. A 5-cm-wide, 2.5-cm-high platform was placed on the floor of the box. During the training session, the rats were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping on the grid, the animals received a 0.4-mA, 2.0-s shock and were returned to their cage. Twenty-four hours after this training session, a retention test identical to the training session but without the shock was performed. The step-down latency during the retention test (maximum = 180 s) was used to measure retention of the inhibitory avoidance memory.[26]

Histologic preparation and analysis

Brain tissue specimens were fixed in 10% formaldehyde and embedded in paraffin. For morphologic examination, 6-μm-thick coronal sections were stained with 3% hematoxylin and eosin. For staining, the sections were deparaffinized with xylene and rehydrated in an alcohol concentration

gradient, followed by rinsing in tap and distilled water. The sections were stained with hematoxylin for 3 to 5 minutes and differentiated with 1% HCl in 70% alcohol. After washing with tap water for 15 minutes, the sections were stained again in eosin for 1 to 4 minutes. This was followed by dehydration and differentiation as described above. The sections were then cleared with xylene, mounted with Permount (Fisher Chemical, Hampton, NH, USA), and observed under a light microscope (BX41; Olympus, Tokyo, Japan) to assess the severity of brain damage at ×400 magnification. The same blinded neuropathologist evaluated the sections for histopathologic signs of damage in the following categories: degeneration of neurons, defined by acidophilic bodies; vascular congestion; and hemorrhage. Pathologic findings in each category were scored using four severity codes: $0 =$ none, $1 =$ mild, $2 =$ moderate, and $3 =$ severe. Each slide was scored, and these scores were averaged for each brain.

Statistical analyses

The software SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analyses. Group size resulting from *a priori* sample size estimation was performed with SigmaStat 3.0 (Systat Software, San Jose, CA, USA) based on estimated differences in the parameters derived from previous studies.[12,13,27] The normality assumption test was performed using the Shapiro–Wilk test. Data obtained from the open field test and physiological parameters were assessed by analysis of variance followed by the Student–Newman–Keuls post hoc test and are expressed as mean ± standard deviation. Data from the inhibitory avoidance test were non-parametric and are reported as interquartile range (25th–75th). In the inhibitory avoidance test,

groups were compared by the Mann–Whitney test. Differences between the training and testing sessions were analyzed by the Wilcoxon test. Differences in cytokines, antioxidant enzymes, MDA, and MPO among the groups were analyzed by two-way analysis of variance, and multiple comparisons were performed by the Newman–Keuls test. A probability value of < 0.05 was considered statistically significant. Array scans were analyzed using the internet-based GEArray Expression Analysis Suite (SuperArray Bioscience Corporation). All genes were normalized to the expression levels of a series of housekeeping genes, and a group of synthetic control sequences was included on the array by the manufacturer. For background normalization, a pair of blank spots and a local background correction for each tetra spot were used. Gene expression was considered significant in the presence of a ≥ 2-fold increase or decrease over the control tissue level.

Results

MAP and blood gas analysis data at baseline and 24, 48, and 72 hours after CLP were compared among the groups. No significant differences in MAP or blood gas values were observed among the groups at all measurement times.

Data regarding serum cytokine levels 72 hours after CLP are shown in Table 1. The TNF-α level was significantly lower in both the CLP + sevoflurane and CLP + isoflurane groups than in the CLP group ($P < 0.05$). The IL-6 and IL-1β levels were significantly lower in the CLP + sevoflurane group than in both the CLP + isoflurane and CLP groups ($P < 0.05$). The IL-1β and IL-6 levels were significantly lower in the sham group than in the CLP and CLP + isoflurane groups ($P < 0.05$).

The antioxidant enzyme activity in all groups is shown in Table 2. The GPX and

Table 1. Systemic inflammatory response detected by serum cytokine release 72 hours after CLP

	TNF-α	IL-1β	IL-6
Sham	41.5 ± 28.6^a	79.8 ± 50.2^b	113.8 ± 70.3^b
CLP	192.0 ± 36.5	250.0 ± 48.2	412.6 ± 89.6
CLP + sevoflurane	93.4 ± 34.3^c	$98.6 \pm 47.3^{c,d}$	$152.7 \pm 3.4^{c,d}$
CLP + isoflurane	86.2 ± 29.8^c	196.1 ± 44.1	323.4 ± 81.6

Data are presented as mean ± standard deviation.
CLP, cecal ligation and puncture; TNF-α, tumor necrosis factor-alpha; IL, interleukin.
[a]$P < 0.05$; sham group compared with CLP ($P = 0.001$), CLP + sevoflurane ($P = 0.02$), and CLP + isoflurane groups ($P = 0.02$).
[b]$P < 0.05$; sham group compared with CLP ($P = 0.003$) and CLP + isoflurane ($P = 0.03$) groups.
[c]$P < 0.05$; CLP + sevoflurane ($P = 0.003$) and CLP + isoflurane ($P = 0.004$) groups compared with CLP group.
[d]$P = 0.003$; CLP + sevoflurane group compared with CLP + isoflurane group.

Table 2. Antioxidant activity in brain tissue detected by GPX, SOD, and CAT 72 hours after CLP

	CAT	GPX	SOD
Sham	2300 ± 320^a	47.9 ± 8.6^b	63.8 ± 11.3^b
CLP	1048 ± 296	11.3 ± 8.4	15.6 ± 9.9
CLP + sevoflurane	1491 ± 288	$35.6 \pm 7.3^{c,d}$	$52.5 \pm 12.1^{c,d}$
CLP + isoflurane	1409 ± 269	22.5 ± 8.1^c	38.6 ± 9.6^c

Data are presented as mean ± standard deviation.
GPX, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; CLP, cecal ligation and puncture.
[a]$P < 0.05$; sham group compared with CLP ($P = 0.002$), CLP + sevoflurane ($P = 0.02$), and CLP + isoflurane groups ($P = 0.03$).
[b]$P < 0.05$; sham group compared with CLP ($P = 0.002$) and CLP + isoflurane ($P = 0.01$) groups.
[c]$P < 0.05$; CLP+ sevoflurane ($P = 0.004$) and CLP + isoflurane ($P = 0.003$) groups compared with CLP group.
[d]$P = 0.01$; CLP + sevoflurane group compared with CLP + isoflurane group.

SOD levels were significantly higher in the CLP + sevoflurane group than in the CLP and CLP + isoflurane groups ($P < 0.05$). The MDA and MPO activities in the brain are shown in Table 3. The serum MDA level was lower in the CLP + sevoflurane and CLP + isoflurane groups than in the CLP group ($P < 0.05$). Moreover, the MDA level was significantly lower in the CLP + sevoflurane group than in the CLP + isoflurane group ($P < 0.05$). Both the CLP + sevoflurane and CLP + isoflurane groups had a significantly lower MPO level than the CLP group ($P < 0.05$).

The results of real-time RT-PCR arrays analysis of the brain tissues are shown in Figure 1. The expression of the proapoptotic genes caspase 3 and caspase 8 was significantly downregulated and the expression of the antiapoptotic genes Bax, Bcl2, and Bcl2l1 (Bclx) was significantly upregulated in the CLP + sevoflurane group compared with the CLP + isoflurane group ($P < 0.05$). The expression of the antiapoptotic genes Bcl2a1 and Bcl2l2 was

Table 3. Lipid peroxidation and neutrophil infiltration in brain tissue detected by MDA and MPO 72 hours after CLP

	MDA	MPO
Sham	24.3 ± 8.2[a]	162.8 ± 36.4[b]
CLP	72.8 ± 9.1	610 ± 92.8
CLP + sevoflurane	29.3 ± 12.2[c,d]	340.8 ± 86.7[c]
CLP + isoflurane	46.3 ± 11.8[c]	386.9 ± 90.9[c]

Data are presented as mean ± standard deviation.
MDA, malondialdehyde; MPO, myeloperoxidase; CLP, cecal ligation and puncture.
[a]$P < 0.05$; sham group compared with CLP ($P = 0.003$) and CLP + isoflurane ($P = 0.02$) groups.
[b]$P < 0.05$; sham group compared with CLP ($P = 0.001$), CLP + sevoflurane ($P = 0.02$), and CLP + isoflurane ($P = 0.02$) groups.
[c]$P < 0.05$; CLP + sevoflurane ($P = 0.002$) and CLP + isoflurane ($P = 0.003$) groups compared with CLP group.
[d]$P = 0.02$; CLP + sevoflurane group compared with CLP + isoflurane group.

significantly upregulated in both the CLP + sevoflurane and CLP + isoflurane groups compared with the CLP group ($P < 0.05$). The activity of caspase inhibitor Birc5 was not significantly different among the groups. The activity of the caspase activator caspase 9 was significantly downregulated in the CLP + sevoflurane group compared with both the CLP + isoflurane and CLP groups ($P < 0.05$).

The open field test and step down inhibitory test results are shown in Figure 2. In the open field test, there were no significant differences in the number of crossings or rearings between the training and test sessions in both the CLP group and CLP + isoflurane group. However, the CLP + sevoflurane group and sham group showed a significant difference in the number of crossings and rearings between the training and test sessions, indicating a significant effect of sevoflurane on the preservation of non-associative memory ($P < 0.05$). In the step down inhibitory test, no statistically significant differences

were found in the latency time between the training and test sessions in the CLP and CLP + isoflurane groups. However, the latency time between the training and test sessions showed a significant difference in the sham and CLP + sevoflurane groups ($P < 0.05$). No significant differences were observed in the latency times of the training sessions among the groups.

The histopathological scores and the wet/dry weight ratio of the four groups are shown in Figure 3. Both sevoflurane ($48.4\% \pm 6.1\%$) and isoflurane ($51.6\% \pm 9.2\%$) resulted in a significant decrease in the wet/dry weight ratio when compared with the CLP group ($77.6\% \pm 3.2\%$) ($P < 0.05$). The histopathology scores were significantly lower in the CLP + sevoflurane and CLP + isoflurane groups than in the CLP group ($P < 0.05$). Representative images of the different categories of damage in each experimental group are shown Figure 4.

Discussion

This study demonstrated that inhalation of volatile anesthetics, particularly sevoflurane, attenuated systemic inflammation and reduced lipid peroxidation, oxidative damage, and apoptosis in the brain tissue in a rat model of CLP. Furthermore, CLP disturbed memory retention and sevoflurane inhalation inhibited this disturbance. The results of this study indicate that these beneficial effects might be attributed to the regulatory effect of sevoflurane on the apoptotic pathway, as indicated by amelioration of antiapoptotic gene expression and blunting of proapoptotic gene expression in the brain tissue of the septic rats.

Sepsis often results in diffuse brain dysfunction without direct central nervous system infection; this condition is defined as SEA and is associated with increased morbidity and mortality.[1–3] Neuroinflammation, neural ischemia,
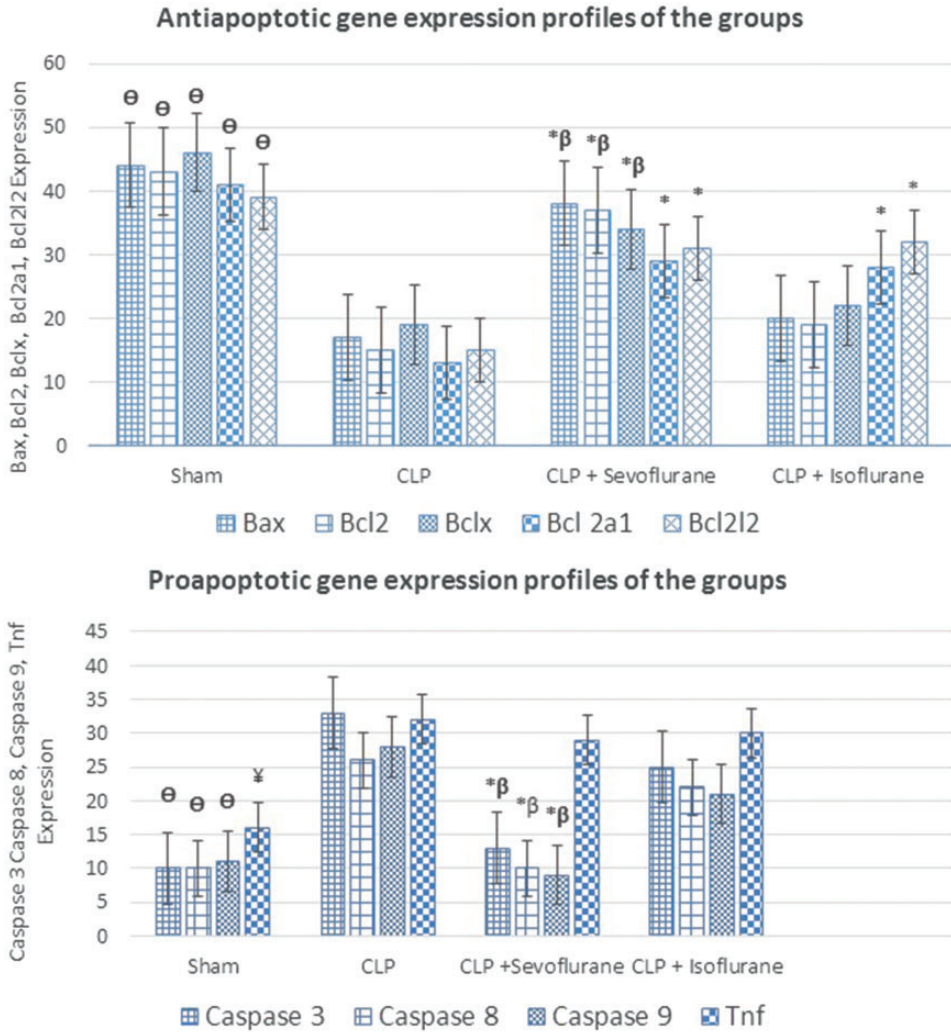
Figure 1. Apoptotic and antiapoptotic gene expression in brain tissue detected by real-time reverse-transcription polymerase chain reaction arrays analysis 72 hours after CLP. *$P < 0.05$; CLP + sevoflurane ($P = 0.001$) and CLP + isoflurane ($P = 0.002$) groups compared with CLP group. $^\beta P = 0.01$; CLP + sevoflurane group compared with CLP + isoflurane group. $^¥ P < 0.05$; sham group compared with CLP ($P = 0.001$), CLP + sevoflurane ($P = 0.02$), and CLP + isoflurane groups ($P = 0.02$). $^\Theta P < 0.05$; sham group compared with CLP ($P = 0.003$) and CLP + isoflurane ($P = 0.01$) groups. CLP, cecal ligation and puncture

neurotoxicity, and apoptosis have been reported as causative factors of SAE, but the underlying pathological changes in SAE are highly complex and multifactorial.[28,29] Systemic inflammation- and sepsis-related production of proinflammatory

mediators is known to have potential neurotoxic effects.[29,30] Bozza et al.[31] showed the association between systemic inflammation and neuronal oxidative damage, neuroinflammation, and neuronal cell death. Neuronal apoptosis in sepsis and its
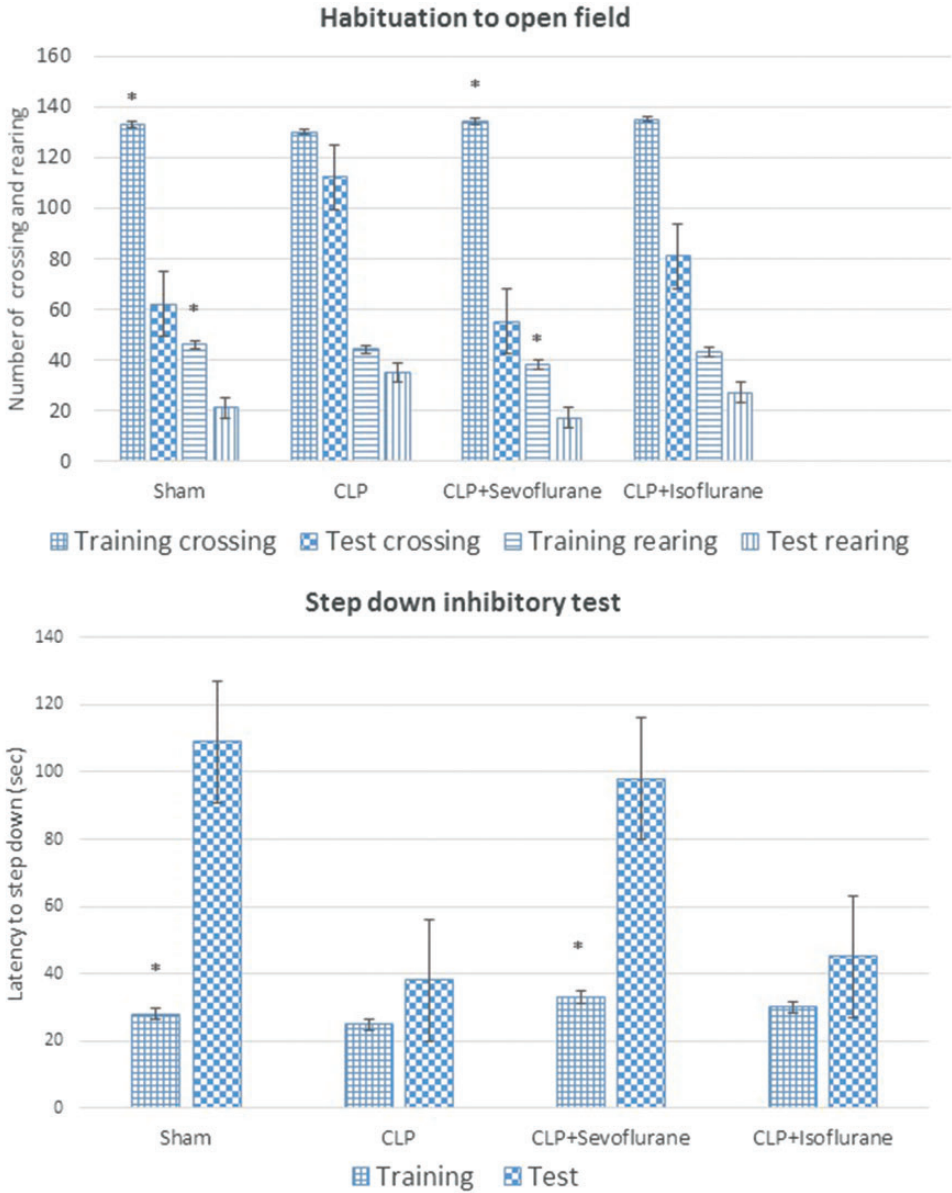
Figure 2. Non-associative and aversive memory evaluated by habituation to an open field test and step down inhibitory test 72 hours after CLP. *$P < 0.05$ when training session is compared with test session. In the open field test, the difference was significant in both the sham group ($P = 0.002$) and CLP + sevoflurane group ($P = 0.003$). In the step down inhibitory test, the latency time showed a significant difference in the sham ($P = 0.001$) and CLP + sevoflurane groups ($P = 0.001$). CLP, cecal ligation and puncture

association with cognitive dysfunction has been well documented. It is conceivable that impairment and apoptosis of neurons in the hippocampus lead to SAE.[14] In the present study, both sevoflurane and isoflurane inhalation effectively suppressed the systemic
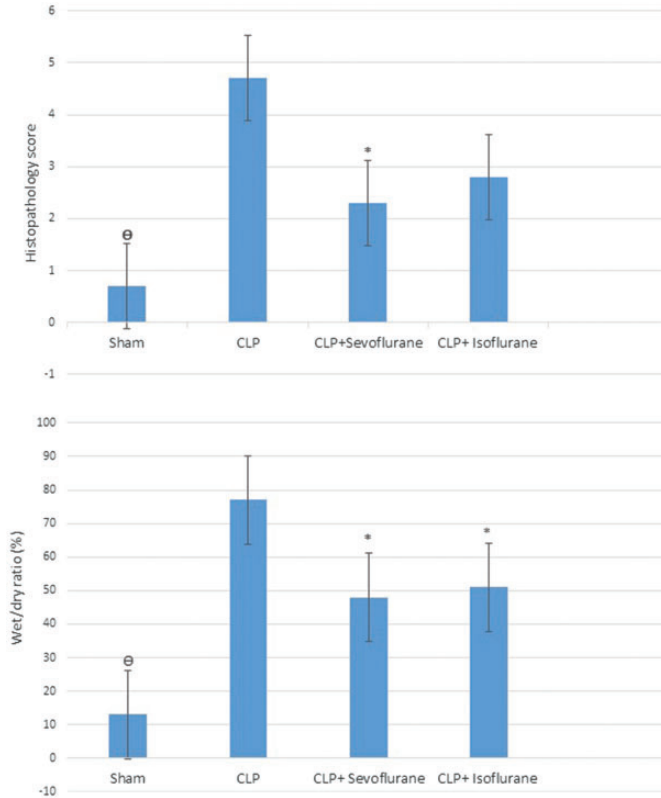
Figure 3. Histopathological scores and wet/dry weight ratio of the four groups. Histopathological scores: *P < 0.05; CLP + sevoflurane (P = 0.01) and CLP + isoflurane (P = 0.02) groups compared with CLP group. ΘP < 0.05; sham group compared with CLP (P = 0.003) and CLP + isoflurane (P = 0.001) groups. Wet/dry weight ratio: *P < 0.05; CLP + sevoflurane (P = 0.002) and CLP + isoflurane (P = 0.003) groups compared with CLP group. ΘP < 0.05; sham group compared with CLP (P = 0.002) and CLP + isoflurane (P = 0.004) groups. CLP, cecal ligation and puncture
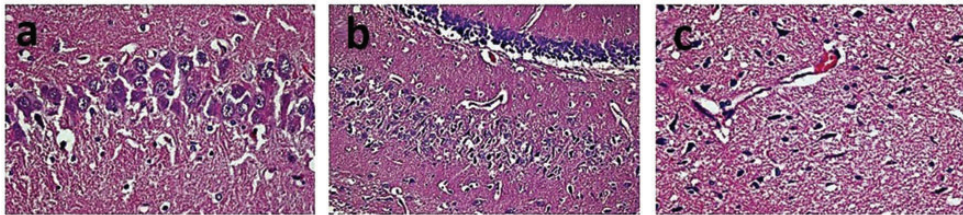
Figure 4. Representative images of the different categories of damage in each experimental group. (hematoxylin and eosin, ×400). (a) A few acidophilic bodies within CA3 segment of Ammon's horn in the sevoflurane group. (b) Many acidophilic bodies within CA4 segment of Ammon's horn in the control group. (c) Vascular congestion and edematous brain tissue in the isoflurane group

inflammatory response caused by CLP. Furthermore, sevoflurane provided an antiapoptotic effect as evidenced by gene expression.

Oxidative stress and mitochondrial dysfunction may occur because of the increased metabolism and energy needed by brain cells secondary to neuroinflammation during sepsis.[32] Mitochondrial dysfunction further causes production of reactive oxygen or nitrogen species, which may result in apoptosis of glial cells and neurons, causing SAE.[32,33] Apoptosis and massive neuronal cell loss leading to brain atrophy is a major mechanism of SEA.[33,34] Several stimuli, including inflammation and cytokines, initiate mitochondria-dependent apoptosis by disturbing the balance between proapoptotic and antiapoptotic proteins.[35] Kim[35] reported that the antiapoptotic Bcl-2 family proteins and BclxL play important roles in inhibiting mitochondria-dependent extrinsic and intrinsic cell death pathways. Previous studies have demonstrated that activation of caspase 2, 8, 9, and 10 plays a role in neuronal apoptosis.[35–37] Semmler et al.[14] investigated how the brain was affected during experimental sepsis and concluded that peripheral inflammation leads to profound glial activation, the generation of nitric oxide, and changes in Bax and Bcl-2 protein regulation critical for apoptosis. In the present study, the apoptotic regulatory effects of sevoflurane and isoflurane were evaluated by detection of apoptotic and antiapoptotic gene expression in septic rats. Our results showed that sevoflurane effectively regulated the expressions of caspase 3, 8, and 9 as well as Bax, Bcl2, and Bclx protein in septic rats.

Other studies have demonstrated an association between brain lesions and long-term psychological or cognitive disorders.[34–38] Barichello et al.[38] showed that with antioxidant treatment, early oxidative damage in the hippocampus and late memory deficits could be attenuated in a rat model of CLP. Shimizu et al.[39] showed that memory retention performance was disturbed 48 hours after the CLP procedure in rats that did not undergo training sessions before the experiment. In the present study, memory impairment was tested 72 hours after sepsis induction, and our results showed that sevoflurane treatment decreased memory impairment. This effect might be attributed to the regulatory effect of sevoflurane on oxidative injury as evaluated by the CAT, GPX, and SOD levels; lipid peroxidation; and apoptotic gene expression in the brain tissue.

Previous studies have shown that systemic inflammation and organ damage improved by volatile anesthetic inhalation in both animals and humans.[40–43] Sevoflurane, a volatile anesthetic agent, exerts neuroprotective effects by reducing cellular injury and decreasing neuronal apoptosis.[44–46] After focal or global cerebral ischemia, sevoflurane postconditioning demonstrates beneficial effects by regulating the expression of apoptosis-related proteins such as Bcl-2 and Bax.[45,47,48] Li et al.[47] showed sevoflurane preconditioning-related protection against cerebral ischemic damage via an antiapoptosis signaling pathway. The results of the present study showed that sevoflurane inhalation effectively enhanced the expression of apoptotic genes, contributing to their beneficial role against memory impairment in a rat sepsis model of polymicrobial peritonitis. Because of these potential beneficial effects, sedation with volatile agents in the intensive care unit is gaining popularity, especially since the approval of the anesthetic conserving device AnaConDa (Sedana Medical AB, Uppsala, Sweden).[17]

In this study, inhalation of the volatile anesthetics isoflurane and sevoflurane attenuated the systemic inflammatory response following CLP in rats. Moreover, sevoflurane inhalation showed a causal link with regulation of apoptotic and antiapoptotic gene expressions, protection against oxidative injury, and inhibition of memory retention in septic rats. Although more studies are needed to characterize the clinical effects of volatile agents, this study provides evidence that sevoflurane sedation in septic patients may have beneficial effects on sepsis-related brain injury and memory impairment.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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