Effects of mid-gestational sevoflurane and magnesium sulfate on maternal oxidative stress, inflammation and fetal brain histopathology

CAGRI OZDEMİR[1], BERRIN ISIK[2], GULCE KOCA[3] and MEHMET ARDA INAN[4]

[1]Department of Anesthesiology and Reanimation, Mamak State Hospital, 06270 Ankara, Turkey; Departments of [2]Anesthesiology and Reanimation, [3]Medical Biochemistry and [4]Medical Pathology, Faculty of Medicine, Gazi University, 06560 Ankara, Turkey

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Abstract. Models of inflammation, oxidative stress, hyperoxia and hypoxia have demonstrated that magnesium sulfate ($MgSO_4$), a commonly used drug in obstetrics, has neuroprotective potential. In the present study, the effects of $MgSO_4$ treatment on inflammation, oxidative stress and fetal brain histopathology were evaluated in an experimental rat model following sevoflurane (Sv) exposure during the mid-gestational period. Rats were randomly divided into groups: C (control; no injections or anesthesia), Sv (exposure to 2.5% Sv for 2 h), $MgSO_4$ (administered 270 mg/kg $MgSO_4$ intraperitoneally) and Sv + $MgSO_4$ (Sv administered 30 min after $MgSO_4$ injection). Inflammatory and oxidative stress markers were measured in the serum and neurotoxicity was investigated histopathologically in fetal brain tissue. Short-term mid-gestational exposure to a 1.1 minimum alveolar concentration of Sv did not significantly increase the levels of any of the measured biochemical markers, except for TNF-α. Histopathological evaluations demonstrated no findings suggestive of pathological apoptosis, neuroinflammation or oxidative stress-induced cell damage. $MgSO_4$ injection prior to anesthesia caused no significant differences in biochemical or histopathological marker levels compared to the C and Sv groups. The present study indicated that short-term exposure to Sv could potentially be considered a harmless external stimulus to the fetal brain.

Introduction

Laparoscopic surgeries, fetal monitoring systems and a better understanding of the pharmacology of anesthetics have increased the safety of surgeries that must be performed with obligatory indications such as acute appendicitis, cholecystectomy and fetal surgery during pregnancy. In the US, 1-2% of all pregnant women undergo surgery for non-obstetric reasons (1) and ~20% undergo surgery for obstetric reasons (2). These surgery rates also indirectly represent the proportion of fetuses exposed to anesthesia. In 2016, the Food and Drug Administration (FDA) issued a statement indicating that repeated or prolonged exposure to inhalation anesthetics during childhood and pregnancy is a potential risk factor for adverse long-term neurocognitive outcomes (3).

Sevoflurane (Sv) is an inhalation agent widely used in obstetric anesthesia due to its clinical advantages. At the receptor level, Sv increases the activity of γ-aminobutyric acid-A and glycine receptors, while decreasing the activity of cholinergic and N-methyl-D-aspartate (NMDA) type glutamate receptors (4,5). However, the effects of this anesthetic agent on the developing brain in different trimesters, at different doses and durations, have not previously been reported, to the best of our knowledge. Despite the widespread use of Sv, certain studies have reported cases of neurotoxicity due to increases in neuroinflammation and oxidative stress, decreases in neuronal transmission, changes in lipid membrane fluidity and apoptosis of neuroprogenitor cells, which suggests that exposure may confer long-term adverse consequences (6-8). A number of reports have indicated that exposure to Sv, particularly during the early and mid-pregnancy periods, can also reduce postnatal learning and memory capacity (9,10). The exposure time and dose are considered the most important determinants of the activation of damage pathways by this anesthetic. However, it has not yet been revealed to what extent the damage will increase as the dose and exposure time increases. Nevertheless, perioperative pharmacological neuroprotection requirements remain a controversial issue.

The neuroprotective efficacy of lidocaine, thiopental, ketamine, propofol, nimodipine, glutamate, aspartate, atorvastatin, erythropoietin and rivastigmine have previously been assessed and reported (11-13). Another drug with potential neuroprotective properties is magnesium sulfate ($MgSO_4$), which has been reported to reduce apoptosis activation and to act as an NMDA receptor antagonist, anti-inflammatory and antioxidant (14-17). Zhang *et al* (18) reported that $MgSO_4$ could ameliorate isoflurane-induced neurotoxicity by

Correspondence to: Dr Cagri Ozdemir, Department of Anesthesiology and Reanimation, Mamak State Hospital, 13 1165th Street, Uregil, Mamak, 06270 Ankara, Turkey
E-mail: mdcagriozdemir@gmail.com

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inhibiting mitochondrial dysfunction, and that it could be used in the prevention and treatment of anesthesia neurotoxicity. Beloosesky *et al* (19) reported that $MgSO_4$ may protect against neuroinflammation by reducing pro-inflammatory cytokine production through an inhibition of neuronal nitric oxide synthase and an NF-κB enhancer. The safety profile of $MgSO_4$, especially in obstetrics, its low cost and its wide availability prompted the present study to explore the neuroprotective efficacy of this agent against possible fetal neurotoxicity caused by Sv.

In the present study, pregnant rats in their mid-gestational period were exposed to short-term, 1 minimum alveolar concentration (MAC) Sv with or without prior $MgSO_4$ injection. The aim of the present study was to evaluate fetal neurotoxicity by measuring changes in maternal inflammation and oxidative stress markers, and analyzing fetal brain histopathology.

Materials and methods

Animals and ethical approval. All procedures were approved by the Gazi University Ethics Committee for Experimental Animals (Ankara, Turkey; approval no. G.U.ET-20-0499). The present study was carried out at Gazi University Faculty of Medicine between September and October 2020. All procedures were performed according to accepted standards in the Guide for the Care and Use of Laboratory Animals (20) and the Animal Research: Reporting of *in vivo* Experiments guidelines (21). The present study included 24 female Wistar albino rats (age, 3-4 months; weight, 200-300 g) supplied by the Gazi University Experimental Animals Research Center. The rats were kept in standard housing conditions, at a temperature range of 20-21˚C, an average humidity of 55±5% and with a 12-h light/dark cycle. Food and water were available *ad libitum*. The rats' pregnancy process was performed and controlled by veterinarians at the research center.

Exposure and treatment. On day 14 of pregnancy, the pregnant rats were randomly assigned and equally divided (n=6) into groups: Control (C), Sv, $MgSO_4$ and Sv + $MgSO_4$ groups. All surgical procedures were performed under general anesthesia. An intraperitoneal (i.p.) injection comprising 50 mg/kg ketamine hydrochloride (500 mg/10 ml Ketalar®; Parke-Davis; Pfizer, Inc.) and 10 mg/kg xylazine hydrochloride (Alfazyne® 2%; Alfasan International B.V.) was administered for anesthesia. The depth of anesthesia was evaluated using the tail-pinch test. Anesthesia was performed in a transparent plastic box.

The rats in Group C were administered O_2 for 2 h on day 14 of pregnancy. The Sv group were administered 2.5% Sv (250 ml; Abbott Laboratories) with 2 l/min O_2 for 2 h on day 14 of pregnancy. The aforementioned concentration of Sv was selected as it corresponded to 1.1 MAC in rats and had been used in previous neurotoxicity studies (10,22). The rats in the $MgSO_4$ group were injected i.p. with 270 mg/kg $MgSO_4$ (15% ampoule; Biofarma Pharmaceuticals) on day 14 of pregnancy. The dosage of 270 mg/kg $MgSO_4$ was selected as it had been reported to show neuroprotective activity in previous studies (14,15,23,24). O_2 at 2 l/min was administered to these rats for 2 h in the plastic box, starting 30 min after the injection. The Sv + $MgSO_4$ group were injected with the same dose of i.p. $MgSO_4$. After waiting for 30 min, the rats were exposed to 2.5% Sv with 2 l/min O_2 for 2 h.

A previous study reported that the same dose of Sv did not change blood pressure and blood gas values; therefore, blood pressure monitoring was not performed in the present study (25). After performing the intervention and exposure steps in each treatment group, a laparotomy was performed under anesthesia in all rats and fetuses were removed.

Pregnant rats and fetuses were sacrificed by taking intracardiac blood under anesthesia. The numbers and weights of fetuses were recorded. The fetal brains were removed by craniotomy. Blood samples (5-10 ml) were taken from the pregnant rats for biochemical analysis. The maternal blood samples were placed in tubes without additives, left to stand upright for 30 min at room temperature and then serum samples were obtained by centrifugation at 1,500 x g for 10 min at 21-23˚C. The resulting serum samples were placed in Eppendorf tubes, frozen at -80˚C and used for subsequent biochemical analysis. The maternal serum samples were used for the determination of inflammatory and oxidative markers. Biochemical analysis could not be performed on the fetal blood, as the samples of intracardiac blood taken from the fetuses for sacrifice were too small for analysis. Brain tissues from 3 rat pups per group were fixed in a 10% formaldehyde solution at room temperature for 48 h. Subsequently, automated routine paraffin tissue processing was performed, followed by staining of 4-micron sections with hematoxylin and eosin. The fetal brain tissues were used for subsequent histopathological analysis of apoptosis, autophagy and neuroinflammation. The timeline of the experiment is shown in Fig. 1.

Biochemical evaluations. The IL-6, IL-10 and TNF-α markers in maternal serum were analyzed using ELISA kits (cat. nos. E-EL-R0015, E-EL-R0016 and E-EL-R2856, respectively; Elabscience Biotechnology, Inc.) according to the sandwich-ELISA principle. The micro-ELISA plates provided in the aforementioned kits were pre-coated with antibodies specific for rat IL-6, IL-10 and TNF-α. Standards or samples were added to the micro-ELISA plate wells and conjugated with the specific antibodies. The rat marker concentrations in the samples were calculated by comparing the optical density value of the samples with those of the prepared standard curve.

The total antioxidant status (TAS) and total oxidant status (TOS) levels in maternal serum were measured using kits (cat. nos. RL0017 and RL0024, respectively; Rel Assay Diagnostics). The serum concentrations of these parameters were assessed following the manufacturer's instructions. TOS levels were expressed as μmol H_2O_2 and TAS levels as mmol Trolox equivalent/l. TOS measurement was performed according to the iron ion-o-dianisidine complex assay. Oxidants present in serum oxidize the ferrous ion-o-dianisidine complex to iron ion. The oxidation is enhanced by glycerol molecules, which are abundant in the reaction medium. Ferric ions, in an acidic environment with xylenol, form an orange-colored complex whose intensity is proportional to the total amount of oxidant molecules present in the sample (26,27).

The ratio of TOS to TAS is considered the oxidative stress index (OSI). The OSI value was calculated according to the

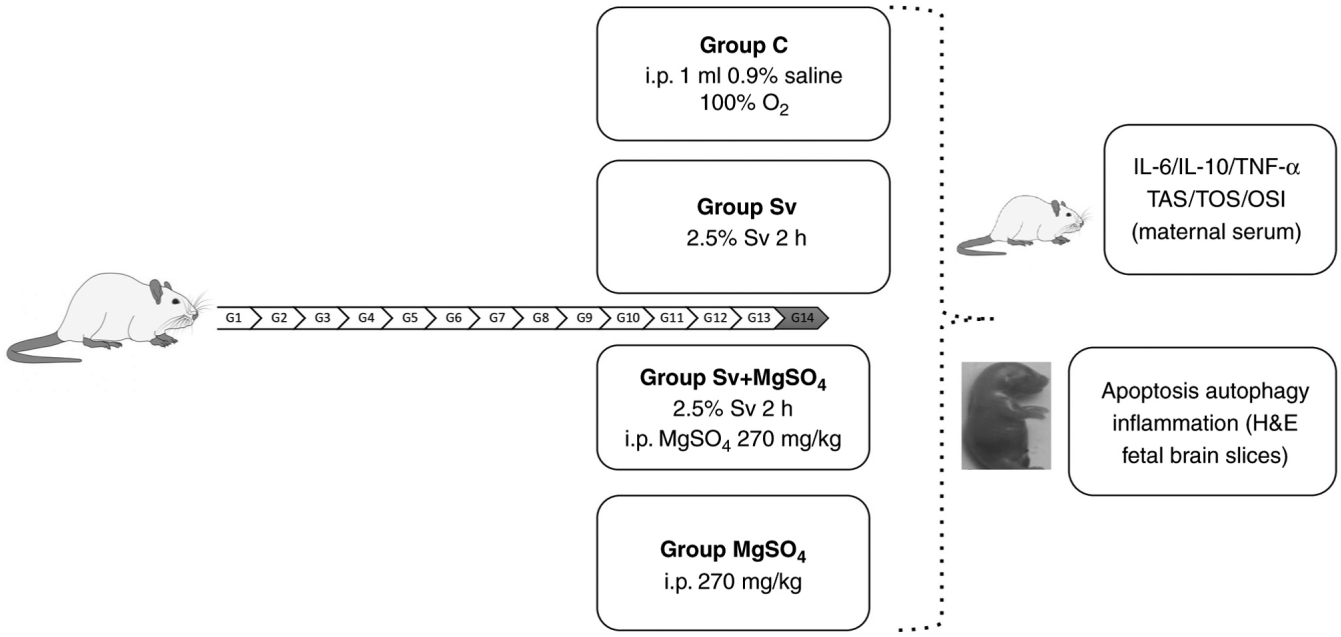

Figure 1. Timeline of the experiment. C, control; Sv, sevoflurane; $MgSO_4$, magnesium sulfate; G, gestational day; H&E, hematoxylin and eosin; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; i.p, intraperitoneal.

following formula: OSI=TOS (μmol H_2O_2 equivalent/l)/TAS (μmol Trolox equivalent/l).

Histopathological evaluation. Fetal brain tissues were evaluated for apoptosis, autophagy and inflammation. All incubations were performed at room temperature. After fixation, the macroscopically visible damaged area was viewed identified in fetal brains sliced into 2-mm sagittal sections. Subsequently, the slice with the widest section surface was loaded into a cassette. Following automated routine paraffin tissue processing with alcohol and xylene solutions, the tissue was embedded in paraffin blocks. For further analysis in the pathology laboratory, sections of 4 μm were cut, deparaffinized and stained with hematoxylin and eosin. The brain samples were imaged using an optical microscope (magnification, x400). Apoptotic cells, identified by shrunken condensed cytoplasm and hyperchromatic nuclei, were examined to evaluate neuronal damage. Cellularity was also assessed to detect inflammatory infiltration. The number of apoptotic cells was quantified in 1 mm^2 areas on each slide, and the highest ratio was recorded for each group. Microscopic images of brain tissue samples from each rat were captured.

Statistical analysis. Statistical analysis was performed using SPSS software (version 15.0; SPSS Inc.). Descriptive statistics were presented as the median (minimum-maximum). Pregnant rat and fetal rat weights, the number of fetal rats taken from each pregnant rat, and maternal serum IL-6, IL-10, TNF-α, TAS, TOS and OSI values were evaluated. The Kruskal-Wallis test was used for analysis of all median values between groups. Parameters that were found to be statistically significant according to the Kruskal-Wallis test were evaluated using a post-hoc test (non-parametric Dunn Bonferroni) to determine between which two groups there was a significant difference. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of midgestational Sv exposure on rat weights. No statistically significant differences in the weights of pregnant rats in the four groups were detected after exposure to Sv (P=0.695; Table I). The numbers and weights of the fetuses taken from each pregnant rat were not significantly different when compared between the treatment groups (P=0.619; Table I).

Effects of midgestational Sv exposure on inflammatory markers. TNF-α, IL-6 and IL-10 levels in maternal serum were evaluated to investigate the potential induction of inflammation as a result of exposure to Sv. TNF-α levels were significantly different when compared between the groups. (P=0.044; Table II). The median TNF-α level of the Sv-treated group was significantly higher when compared with the control group (P=0.027; Table III). No significant differences were demonstrated in other pairwise comparisons of the other inflammatory marker levels between the treatment groups.

Effects of midgestational Sv exposure on oxidative stress markers. The oxidant-antioxidant stress markers TAS, TOS and OSI were evaluated in serum samples from pregnant rats. These results demonstrated no significant differences between the groups (TAS, P=0.153; TOS, P=0.256; and OSI, P=0.258; Table IV).

Histopathological evaluation. The morphology of apoptotic cells can be described as showing cytoplasmic constriction, eosinophilic condensation and nuclear hyperchromasia (28). The number of apoptotic cells per 1 mm^2 was determined by examining cortical regions following the initial observation of apoptosis and counting apoptotic cells in consecutive high-magnification fields (Table V). A 1-mm^2 section corresponded to ~4 high-magnification fields of view (each

Table I. Weights of pregnant rats and weights and numbers of fetuses in the treatment groups.

Parameter	Control group (n=6)	$MgSO_4$ group (n=6)	Sv group (n=6)	Sv + $MgSO_4$ group (n=6)	P-value
Weight of pregnant rats, g	260.87 (247.52-295.51)	252.34 (240.91-284.24)	260.19 (239.19-280.13)	262.08 (250.13-290.64)	0.695
Fetuses, n	7.5 (3.0-11.0)	6.0 (3.0-10.0)	7.0 (3.0-9.0)	7.0 (4.0-10.0)	0.906
Weight of fetuses, mg	140.78 (130.42-151.98)	145.78 (137.65-150.62)	141.38 (139.45-147.43)	142.45 (138.25-146.52)	0.619

Values are expressed as the median (range). Sv, sevoflurane; $MgSO_4$, magnesium sulfate.

Table II. Comparison of inflammation marker levels between treatment groups of rats.

Marker	Control group (n=6)	$MgSO_4$ group (n=6)	Sv group (n=6)	Sv + $MgSO_4$ group (n=6)	P-value
TNF-α, pg/ml	71.94 (58.96-111.32)	87.35 (63.95-263.46)	159.64 (96.37-261.35)	95.07 (63.12-206.79)	0.044
IL-6, ng/l	2.69 (2.39-3.48)	2.87 (2.38-3.23)	2.88 (2.70-3.53)	2.85 (2.47-3.72)	0.492
IL-10, pg/ml	129.61 (66.88-176.90)	160.44 (100.36-215.70)	138.22 (100.36-220.71)	203.08 (79.30-274.56)	0.534

Values are expressed as the median (range). Sv, sevoflurane; $MgSO_4$, magnesium sulfate.

Table III. Comparison of TNF-α levels between treatment groups of rats.

Comparison	Test statistic	Standard error	Standard test statistic	P-value	Adjusted P-value
C vs. $MgSO_4$	-5.417	4.082	-1.327	0.184	>0.999
C vs. Sv + $MgSO_4$	-6.000	4.082	-1.470	0.142	0.849
C vs. Sv	-11.583	4.082	-2.838	0.005	0.027
Sv + $MgSO_4$ vs. $MgSO_4$	-0.583	4.082	-1.43	0.886	>0.999
$MgSO_4$ vs. Sv	-6.167	4.082	-1.511	0.131	0.785
Sv vs. Sv + $MgSO_4$	5.583	4.082	1.368	0.171	>0.999

Bonferroni's correction was used to control for multiple comparisons and were shown with adjusted P-values. C, control; Sv, sevoflurane; $MgSO_4$, magnesium sulfate.

0.26 mm^2). Brain sections of the different treatment groups were examined to search for macroscopic areas showing necrosis, gliosis and inflammation that could be associated with inflammation, oxidative stress or apoptosis. The histopathological images were similar between the groups and no evidence, such as inflammation, pathological apoptosis and autophagy, was found to suggest anesthesia-induced damage. Although apoptosis was particularly concentrated in the periventricular adjacent white matter areas in all examples, the extent of damage was considered to be within the limits of physiological apoptosis normally seen during embryonic development (29) (Fig. 2). When the number of apoptotic cells was compared between treatment groups, the group with the highest median value was the Sv + $MgSO_4$ group, followed by the $MgSO_4$, Sv and C groups, respectively. However, the differences in the number of apoptotic cells between the groups were not statistically significant (P=0.114; Table V).

Discussion

Medical developments such as laparoscopy and intrauterine fetal monitoring have enabled the performance of emergency interventional procedures during pregnancy in a safer manner. However, surgical procedures have increased the rate of exposure of the fetal brain to anesthesia (30). The net effect of anesthetic drugs on the fetal brain is a long-debated issue and previous studies have provided conflicting results (10,31,32). In 2016, the FDA reported that repeated or prolonged use of general anesthetic and sedation drugs in children aged <3 years, or in pregnant women in the third trimester, may negatively affect children's brain development (3).

The first step in reducing fetal risk under anesthesia is the timing of the surgical procedure. Currently, the second trimester is accepted as the most appropriate time for mandatory surgeries (33). The present study aimed to contribute to the ongoing debate by focusing on the acute damage that could

Table IV. Comparison of oxidative stress markers between treatment groups of rats.

Marker	Control group (n=6)	$MgSO_4$ group (n=6)	Sv group (n=6)	Sv + $MgSO_4$ group (n=6)	P-value
TAS, mmol/l	1.60 (1.45-1.81)	1.64 (1.42-3.15)	1.78 (1.63-2.35)	1.57 (1.47-180.00)	0.153
TOS, μmol/l	4.99 (3.65-14.44)	7.26 (2.38-19.77)	9.49 (5.97-67.95)	11.43 (3.28-25.73)	0.256
OSI	0.30 (0.24-0.79)	0.49 (0.09-1.10)	0.55 (0.35-2.89)	0.75 (0.21-1.42)	0.258

Values are expressed as the median (range). Sv, sevoflurane; $MgSO_4$, magnesium sulfate; TAS, total antioxidant status; TOS, total oxidant status.

Table V. Comparison of number of apoptotic cells in fetal brain tissues between treatment groups of rats.

Parameter	Control group (n=6)	$MgSO_4$ group (n=6)	Sv group (n=6)	Sv + $MgSO_4$ group (n=6)	P-value
Apoptotic cells, n	1.0 (1.0-3.0)	4.0 (2.0-11.0)	2.5 (2.0-4.0)	4.5 (3.0-7.0)	0.114

Values are expressed as the median (range). Sv, sevoflurane; $MgSO_4$, magnesium sulfate.

potentially occur to the fetus following exposure to anesthesia in the second trimester. Day 14 of rat pregnancy (G14) was selected, as it is equivalent in terms of fetal brain development to the second trimester development that occurs during a human pregnancy (34,35).

Other risk-reducing steps include limiting the exposure time, frequency and drug dose. Sv, as the most frequently preferred volatile anesthetic agent for pregnant women (36), was used at a concentration of 2.5% in the present study. The possible neurotoxic effects of Sv have been reported in a number of studies (8,37), but its actual neurotoxic potential, if present, has not yet been reported, to the best of our knowledge. The 2.5% concentration of Sv is equivalent to 1.1 MAC in rats (4). A time frame of 2 h was chosen, as current recommendations are that surgeries should be performed in as short a time frame as possible to reduce exposure. A previous study reported that a 2-h anesthetic exposure was sufficient to trigger neurotoxicity (38). The present study was designed considering current recommendations for clinical approach and operation times, unlike other previously published studies that aimed to induce neurotoxicity using high Sv doses, long exposure times and repeated applications (39,40).

In the present study, Sv did not cause neurotoxicity in the fetal rat brain. Similar previous studies also support this result. For example, Wu *et al* (32) reported no neurotoxicity in second trimester pregnant rats after a 2-h exposure to Sv and emphasized the importance of repeated exposures in the development of neurotoxicity. Furthermore, Lee *et al* (31) reported that neither single nor multiple exposures to Sv caused any long-term behavioral disorders and that they did not affect long-term synaptic plasticity. By contrast, learning disabilities and neurotoxicity in offspring rats were reported by Zheng *et al* (10) following exposure to 2.5% Sv for 2 h and by Hirotsu *et al* (39) following exposure to 1-2% Sv for 3 h. The methods used by the aforementioned studies were similar to those used in the present study; therefore, the differences in results may be due to the species choice (mouse or rat), anesthesia protocol (exact gestational age, choice of anesthetic agent, dose and duration), sensitivity of the biochemical tests and the chosen exposure time point (fetal or neonatal). In the present study, the histopathology of the fetal brain and the inflammatory and oxidative stress markers in maternal blood samples were assessed for the fetal neurotoxicity evaluation. Maternal cytokines and radicals can reach the fetal brain through the placenta and move across the immature fetal blood-brain barrier, thereby producing proinflammatory cytokines that activate fetal microglia and impair neuronal development (41).

Neuroinflammation is a pathological condition that can lead to cognitive impairment (42). Previous studies have reported an association between elevated cytokines in maternal serum in the second and third trimesters of pregnancy and an increased risk of neurodevelopmental disorders (34,39,43). Possible mechanisms that could be responsible for this association are that the increased cytokines in the maternal blood reach the brain across the immature blood-brain barrier (44) or that Sv directly activates NF-κB to increase levels of proinflammatory cytokines in the fetal brain (37). In the present study, only the maternal serum levels of TNF-α demonstrated a significant increase, but this increase did not appear to cause fetal brain damage. In another previous study investigating the effects of maternal inflammation on the fetal brain, L-6, IL-1β, IL-10, TNF-α, TNF-α levels were the first to increase among the cytokines tested (44). The effects of TNF-α on cognitive function, disease occurrence and underlying disease have been investigated previously (45). Under pathological conditions, microglia secrete TNF-α, an important component of neuroinflammation. TNF-α may potentiate cytotoxicity through two complementary mechanisms: Indirectly by inhibiting glutamate transport by astrocytes and directly by increasing the localization of ionotropic glutamate receptors on synapses (46). Acute inhibition of endogenous TNF-α by treatment with soluble TNF-α receptors, neutralizing antibodies or antisense blockers reduces ischemic and traumatic brain damage in rodents, which suggests that TNF-α contributes to brain damage (47-50). TNF-α induces caspase-3 activation

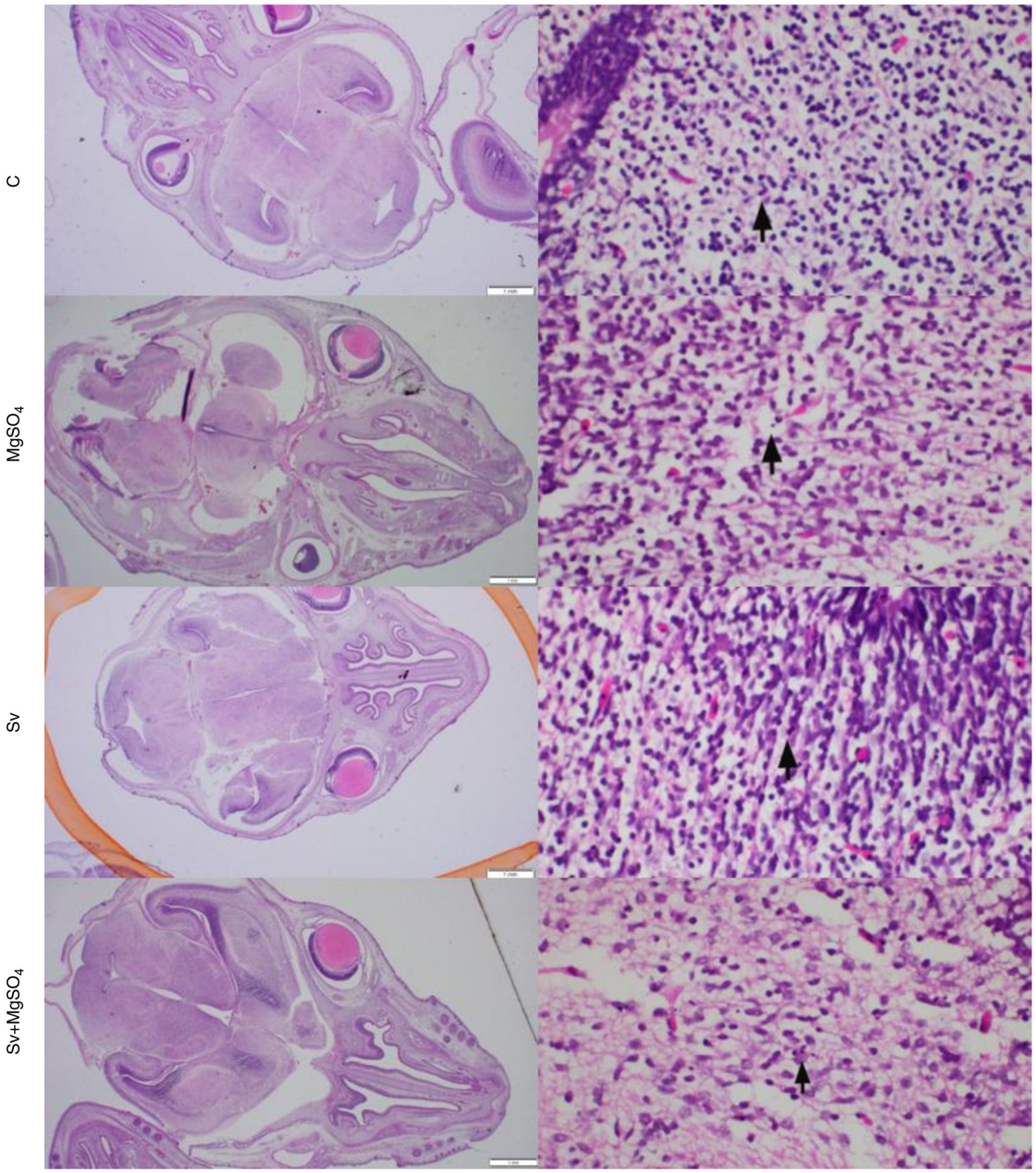

Figure 2. Sagittal sections of hematoxylin and eosin-stained fetal rat brain tissue at day G14. Left panel: Magnification, x12.5; scale bar, 1 mm. Right panel: Magnification, x400. Black arrows indicate apoptotic cells. C, control; Sv, sevoflurane; $MgSO_4$, magnesium sulfate.

that causes apoptotic neuronal cell death in hippocampal cultures (51). A number of studies have also reported that TNF-α is involved in mediating microglia-induced neuronal cell death (52) and peripherin-induced dorsal root ganglion neuron apoptosis (53). TNF-α alone has also been reported to induce oxidative stress that may lead to cell death (54,55). However, an increase in TNF-α levels does not necessarily mean that neurotoxicity has occurred. The minimum level of TNF-α that can cause neurotoxicity is currently unknown. While a single dose of TNF-α did not damage the memory of healthy mice, it caused acute disturbances such as hypothermia, weight loss and inactivity in mice with neurodegeneration. However, no neuronal death, synaptic loss or tau hyperphosphorylation was observed in the brain tissue (44). Similarly, a 3% Sv exposure

for 2 h did not increase IL-6 and TNF-α levels in 6-day-old rat pups, whereas treatment with 3% Sv for 2 h daily for 3 days increased IL-6 and TNF-α levels in the brain (37). In terms of neuroinflammation, repeated exposure and prolonged exposure times to Sv may cause increased inflammation. The increased levels of IL-6 and IL-10 reported in previous studies are most likely associated with higher doses, longer durations and repeated exposures. However, more extensive studies are needed to confirm this.

Compared with the adult brain, the developing brain shows a higher rate of mitochondrial respiration and oxygen consumption. However, the fetal brain has insufficient antioxidant defenses compared with the adult brain, making it more susceptible to the effects of oxidative stress (56,57). Nevertheless, whether Sv affects the fetal brain oxidative balance positively or negatively currently remains unclear. As the Sv concentration increases, the antioxidant activity shifts to an effect that increases oxidative stress (58). At present, the safety of using the 1 MAC Sv concentration frequently used in the clinic remains unclear, as different studies have reported both antioxidant and pro-oxidant effects at this dose. Zhou *et al* (7) reported relatively low plasma and hippocampal malondialdehyde (MDA) levels of Sv at a subclinical concentration of 0.3 MAC (1.3%) and reported that Sv could reduce oxidative stress. Additionally, Allaouchiche *et al* (59) investigated the oxidative state of the circulation and lungs during 1 MAC Sv anesthesia, and observed relatively lower levels of MDA and glutathione peroxidase in plasma and bronchoalveolar lavage fluid compared with levels during propofol and desflurane anesthesia; therefore indicating the antioxidant effect of Sv. Conversely, a study comparing surgeries performed with propofol anesthesia and Sv showed that erythrocyte protection was impaired during Sv anesthesia, which indicated that Sv may cause oxidative stress (60). Exposure to Sv in newborn rats has been reported to increase reactive oxygen species (ROS) levels through mitochondrial dysfunction and overactivation of NADPH oxidase, resulting in widespread neurodegeneration and long-term behavioral disorders (10,57,61). Exposure-induced intracellular accumulation of ROS has been associated with neuronal apoptosis (62,63). Studies examining the effect of anesthesia exposure on oxidative stress during pregnancy are limited. A previous study reported that 6 h of 3% Sv anesthesia in 6-day-old newborn rats caused oxidative stress by decreasing superoxide dismutase levels and increasing MDA levels in the rat brain (40). Liu *et al* (62) reported the same result in 7-day-old rats. However, the anesthesia exposure time used in the aforementioned studies was not compatible with Sv use in clinical practice, as the applied Sv concentration was >1.1 MAC. The results reported in the present study suggested that the application of Sv anesthesia at 1.1 MAC for 2 h did not cause acute biochemical or pathological oxidant damage in fetal rats.

Physiological apoptosis is responsible for the destruction of 50-70% of neurons that develop under normal conditions. Exposure to anesthetics at toxic levels, which may occur during these periods when neuronal development is at the forefront, can increase the rate of physiological apoptosis to pathological levels (64). Anesthesia can increase the occurrence of this physiological process to a pathological level (8,37,65,66) and previous studies have shown that certain areas of the brain can be more strongly affected (67,68). The hippocampus has been widely studied, as it is the key structure for spatial memory and learning. However, Satomoto *et al* (68) observed apoptosis in numerous regions of the newborn rat brain immediately after exposure to Sv. Therefore, a holistic viewpoint in histopathological evaluation may provide more accurate results of the damage caused by this anesthetic. However, examining tissues that have not completed their development and whose borders are not clear is often difficult. In the present study, the total apoptotic cell count in the cortex was used as a measure for histopathological evaluation, as the early gestational period (G14) was optimal in terms of brain development. The results of the present study did not indicate any adverse histological pathological findings of the effects of anesthesia exposure in the second trimester in the fetal brain. However, a previous study that investigated the effects of 3.5% Sv for 2 h in G14 rats reported increased apoptosis in neural stem cells and adverse effects on behavioral tests, but these results were not observed when the concentration of Sv was decreased to 2% (69). Although apoptosis induced by the high dose of Sv gradually decreased as the rat fetuses progressed further towards the neonatal period, the results of the behavioral tests did not improve. In the present study, which was similar to the aforementioned study in terms of exposure time point and duration, the occurrence of apoptosis was attributed to the high dose of Sv administered.

The current consensus is that not every apoptotic process that may occur after exposure to anesthetics will have long-term consequences (66). Cognitive losses resulting from increased apoptosis may be compensated for during development, but this process can be unpredictable. Exposure to 3-5% Sv for 6 h in 6-day-old rats (70) and 2.5% Sv for 4 h in 7-day-old rats did not cause neuronal loss (71), which indicates that the risk of damage is lower when exposure is delayed. A previous study investigated repeated exposures to 3% Sv in G14 rats, and reported neuronal cell loss and long-term cognitive impairment accompanied by a decrease in histone acetylation in fetal brain tissue and a decrease in brain-derived neurotrophic factor levels (72).

These previous findings highlight the importance of perioperative pharmacological neuroprotection, but this remains a controversial topic. However, in patient groups where multipharmacy is avoided, such as pregnancy and pediatrics, it would be a more rational approach is to administer a drug with a proven safety record that is in frequent use, rather than opting to use a new agent. In previous years, the neuroprotective activity of $MgSO_4$ had been reported, which is frequently used in preterm labor and is familiar to most obstetric anesthesiologists (73) and to obstetric clinicians who use it to prevent preterm labor and eclamptic seizures. A number of studies have reported that $MgSO_4$ protects the central nervous system from oxidative stress and ischemic events, and reduces neonatal learning and memory problems caused by maternal inflammation (15,74). However, to the best of our knowledge, the relationship between Sv and magnesium has not previously been reported in neurons, as previous studies have been limited to analgesia and hemodynamics. The aim of the present study was determined by a review of the literature, which indicated that magnesium could suppress the damage pathways induced by Sv (14-17).

Sv induces neuroinflammation by activating the NF-κB signaling pathway, whereas magnesium inhibits the activation of NF-κB and reduces levels of pro-inflammatory cytokines, including TNF-α, IL-1α and IL-6 (75). Sv increases cytosolic calcium levels, which results in abnormal calcium release from the endoplasmic reticulum. Increased intracellular calcium serves a role in stimulating mitochondrial ROS production and inducing cytokine release (76). Magnesium, by contrast, is a calcium uptake antagonist (77); therefore, it reduces the acquisition of the neurotoxic phenotype by microglia (78). Microglia overproduce ROS, which serve a role in neurodegeneration (79), and Sv has also been reported to increase ROS formation. Magnesium reduces ROS production (80). In the brain, magnesium has been reported to reduce oxidative damage after hypoxia (81), counteracting oxidative stress caused by maternal inflammation (79). For this reason, the present study investigated the neuroprotective effect of $MgSO_4$, administered at 270 mg/kg i.p. 30 min before Sv exposure, as a possible suppressor of Sv-induced neurotoxicity. Han *et al* (14) reported that $MgSO_4$ at 270 mg/kg i.p. reduced oxidative stress and inflammation after intrahepatic cholestasis in pregnant rats. Khatib *et al* (15) showed that the same dose reduced inflammation-induced fetal brain damage in maternal late gestational inflammation. A previous study reported that anesthetics increased cytosolic calcium levels and elevation of cytosolic calcium was associated with increased levels of proinflammatory cytokines, potentially through activation of the NF-κB signaling pathway (82). Magnesium serves a neuroprotective role by preventing the entry of cytosolic calcium; however, Dribben *et al* (83) reported the occurrence of neuroapoptosis after high-dose magnesium administration in neonatal mice.

Magnesium has complex effects on cellular excitability, including both stimulatory and inhibitory effects; it has also been reported to induce neuronal apoptosis *in vivo*. These two responses are considered to reflect the direct effects of magnesium on sensitive neurons rather than secondary consequences of systemic magnesium administration to rats (79). In clinical obstetrics, $MgSO_4$ administration is typically titrated to maintain maternal serum levels at 4-8 mg/100 ml (1.6-3.3 mM) (83). These concentrations are close to the lowest concentrations that caused the significant neuronal toxicity reported in previous studies (83). Therefore, in the present study, the neurotoxic potential of $MgSO_4$ was analyzed, as well as its potential neuroprotective activity. In the present study, no significant difference in inflammatory and oxidative stress markers were demonstrated between the treatment groups, except for the increased TNF-α levels in the Sv group. The TNF-α levels in the $MgSO_4$-treated groups were similar compared with those in the control group, which suggested the anti-inflammatory activity of $MgSO_4$. Since the IL-6, IL-10, TAS, TOS and OSI levels were not statistically different between groups, no positive or negative effect can be attributed to $MgSO_4$ in terms of these markers. However, the fact that the number of apoptotic cells in the $MgSO_4$-treated groups was markedly higher compared with the other groups supports the findings reported by Dribben *et al* (83). Although the efficacy of the dose applied in the present study has been confirmed in previous studies, lower doses should be tested in future studies. Other injury pathways are implicated in anesthesia-induced neurotoxicity. Autophagy, parthanatos, decreased excitatory synapses and disruption of synaptic plasticity are other pathways that may be affected by anesthesia and require further investigation.

The present study had certain limitations. The first was the lack of hemodynamic monitoring of rats during Sv exposure. Wang *et al* (84) showed that hemodynamics and blood gas parameters in rats exposed to 2 and 3.5% Sv were similar compared with the control group. Similarly, Li *et al* (85) reported that the blood pressure, heart rate, pH, arterial carbon dioxide tension, arterial oxygen tension and arterial oxygen saturation of Wistar Albino rats exposed to 2.6% Sv for 4 h were no different from those of the control group. The laboratory where the experiments were performed does not have the necessary devices for hemodynamic monitoring. At the same time, a previous similar study (25) showed that exposure to Sv did not change hemodynamic parameters in Wistar rats and that the study could be performed successfully without monitoring. These results eliminated concerns in the present study about the possible hemodynamic adverse effects of Sv. A second limitation of the present study was the use of 100% O_2 during anesthesia to avoid hypoxemia. High O_2 concentrations have been reported to be harmful, but their application to every treatment group, including the control, minimized the effect on the results of the present study. This methodology was selected so that the respiratory functions of the rats did not deteriorate and the rats did not die during the experiment. In order to prevent this limitation, the oxygen saturation of rats, partial O_2 and CO_2 pressures, and inlet and outlet gas levels of gases in the closed environment should be monitored. Only in this way can the possible toxic effects of oxygen be avoided by giving the monitor as much oxygen as necessary.

In conclusion, 2 h exposure to Sv at a concentration of 1.1 MAC did not cause maternal inflammation, oxidative stress or neuronal damage in the fetal brain. The increase in TNF-α levels may indicate that this exposure level may trigger inflammation, although it may be too low to cause damage. The findings of the present study suggested that short-term administration of Sv could be used safely in the second trimester, based on the results of a rat model. Although there appear to be potential anti-inflammatory and antioxidant activities of $MgSO_4$, more comprehensive studies are required to confirm any neuroprotective benefits due to concerns about the potential for inducing apoptosis.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CO and BI designed the study and performed the experiments. CO collected samples. CO, BI, GK and MAI confirm the authenticity of all the raw data. GK performed the biochemical assessments. MAI performed the histopathological analysis of brain tissue. CO analyzed and interpreted data. CO and BI drafted the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Ethical approval for the study was obtained from Gazi University Experimental Animals Ethics Committee (Ankara, Turkey; approval no. G.U.ET-20-0499).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Dr Cagri Ozdemir, ORCID no. 0000-0002-1266-8054; Professor Berrin Isik, ORCID no. 0000-0002-0420-6589; Dr Gulce Koca, ORCID no. 0000-0002-2646-1003; and Dr Mehmet Arda Inan, ORCID no. 0000-0002-6179-2828.

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