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Original Article

Sevoflurane induces apoptosis of isolated placental trophoblast cells and stimulates expressions of TNF-α and IL-6

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Abstract: Studies have shown that narcotic drugs may affect the function of placental trophoblast cells. The aim of this study was to investigate the effect of sevoflurane on apoptosis and proinflammatory cytokines in isolated placental trophoblast cells. The primary placental trophoblast cells were obtained from a total of 20 parturients, which were randomly divided into 4 groups and treated with 3% sevoflurane for 0 minutes (S0), 15 minutes (S15), 30 minutes (S30) and 60 minutes (S60). The expressions of CK7 and vimentin were detected by immunofluorescence. The apoptosis of trophoblast cells was tested by TUNEL assay. The concentrations and protein expressions of TNF-α and IL-6 were determined by ELISA and Western-blot. The apoptosis number and apoptosis rate of placental trophoblast cells in S60 and S30 groups were higher than that in S15 and S0 groups ($P<0.05$). The concentrations of TNF-α and IL-6 in cell culture medium of S60 and S30 groups were elevated as compared to S15 and S0 groups ($P<0.05$). Compared with S15 and S0 groups, the protein expressions of TNF-α and IL-6 in placental trophoblast cells of S60 and S30 groups also showed an significant increase ($P<0.05$). Moreover, the expressions of TNF-α and IL-6 were positively correlated with the apoptosis of cytotrophoblast cells. Using for a long time of sevoflurane induces the apoptosis of placental trophoblast cells and increases the expressions of pro-inflammatory factors, suggesting that the duration of sevoflurane anesthesia should be controlled within 15 minutes.

Keywords: Sevoflurane, placental trophoblast cells, apoptosis, interleukin-6, tumor necrosis factor-α

Introduction

The Food and Drug Administration (FDA) of US points out that general anesthesia by some narcotic drugs can affect the nervous system development of infants [1] due to general anesthetics can be transmitted through the placental barrier and transported to the fetal blood circulation system. So the risks and benefits of using the anesthetic during pregnancy to the fetus and pregnant women should be weighed [2]. The integrity of the placental function and structure is essential for the growth and development of the fetus and for maintaining the stable physiological state of maternal body [3]. Therefore, research on perioperative placental function and structure has become a hot topic. Sevoflurane is a new inhalation anesthetic that is currently widely used in clinical practice. It is characterized by rapid onset of anesthesia, perfect block, good muscle relaxation, low dosage, good smell, and rapid and smooth induction [4-6]. However, the effects of sevoflurane on the structure and function of placental trophoblast cells are not clear. In this study, we used different time gradients of sevoflurane to interfere with isolated placental trophoblast cells and determined their effects on placental trophoblast apoptosis and proinflammatory cytokine expression, providing some scientific data for the safe use of sevoflurane in general anesthesia in obstetrics.

Materials and methods

Ethics statement

The study was registered at the China Clinical Trial Registration Center (Registration No.: ChiCTR1800014571) and an ethical review

Table 1. Comparison of general data of parturients (X±S)

Items	Grouping of placental trophoblast intervention time based by sevoflurane (5 cases each group)				*P* value
	0 minute (S0)	15 minutes (S15)	30 minutes (S30)	60 minutes (S60)	
Age (years)	30.1±3.0	29.5±2.7	29.5±2.9	30.1±3.0	>0.05
Gestational times (weeks)	38.9±2.1	37.1±0.9	36.9±3.1	37.8±2.1	>0.05
BMI of parturients (kg/m^2)	22.4±1.5	22.9±1.8	23.2±2.5	21.5±1.9	>0.05
Dose of ephedrine (mg)	5.6±2.3	6.1±1.5	6.6±3.3	5.9±3.5	>0.05
Scar uterus (n, %)	3, 60	4, 80	3, 60	4, 80	>0.05
Abnormal fetal position (n, %)	1, 20	1, 20	1, 20	0, 0	>0.05

X±S, mean ± standard deviation; BMI, body mass index.

was submitted to the hospital ethics committee and approved (Project Approval Number: 201701080). All participants have been fully informed of the purpose and plan of the study and signed a written informed consent form.

Participants

From October 2017 to May 2018, a total of 20 women who underwent cesarean section under spinal anesthesia were included in the study (Department of anesthesiology, Guangdong Women and Children Hospital, Guangzhou, Guangdong, China). Detailed clinical data of participants are shown in **Table 1**.

Inclusion and exclusion criteria

Inclusion criterion: (1) pre-anesthetic assessment of the women is graded I to II using the American Academy of Anesthesiologists (ASA) grading system [7]; (2) no history of diabetes, gestational hypertension, and neurological diseases; (3) no fetal development delay, congenital malformation, placenta previa; premature rupture of membranes, premature birth and signs of infection; and (4) meeting the indications of cesarean section operation according to the criterion made by Obstetrics group, Obstetrics and Gynecology, Chinese Medical Association [8].

Exclusion criteria

Exclusion criteria: (1) women combined with serious surgical diseases; (2) twins and multiple births; (3) with esophageal hiatal hernia and gastroesophageal reflux disease history; (4) fetal distress; (5) using any sedative, analgesic and anti-inflammatory drugs within 2 hours before surgery; (6) liver and kidney function, electrolyte abnormalities of women; and (7) fetal weight >2500 g.

Specimen collection

After the fetus was delivered, the removed placenta was placed in a sterile tray. Placental tissues with an area of approximately 1 cm^3 were cut off under aseptic conditions and fetal membranes, placental tissues with calcification, hemorrhage, infarction and blood vessels were removed. The placental tissues harvested were immediately rinsed 3 to 4 times with pre-cooled sterile phosphate buffered saline (PBS).

Isolation of placental primary trophoblast cells

The minced placenta tissue was digested twice in dulbecco's PBS with 0.125% trypsin and 0.1 mg/mL deoxyribonuclease I at 37°C for 20 minutes each time. The released cells were first filtered through a 40 μm filter (Thermo Scientific), and an equal amount of DMEM medium containing 10% fetal bovine serum, 100 u/mL penicillin, and 100 μg/mL streptomycin were added. The cell suspension was collected and centrifuged at 2400 rpm for 10 minutes. The cells were resuspended in 3 mL of dulbecco's PBS, layered by Percoll gradients (70%, 50%, 30% and 20%; 5 mL each) and centrifuged at 400 rpm for 25 minutes at 4°C. A total of 50 mL of suspension was taken from 30% to 50% of Percoll trophoblast cells (density: 1.048 to 1.060 g/mL) and centrifuged at 2400 rpm for 5 minutes at 4°C. After the supernatant was removed, the obtained cells were resuspended in DMEM medium containing 20% fetal bovine serum and counted using a hemocytometer plate.

Identification of primary trophoblast cells of placenta

To identify the placental trophoblast cells, the expression levels of the marker proteins CK7 (cytokeratin-7, 1:100, Ab181598, US AbCAM)

and vimentin (Vimentin, 1:250, Ab92547, US AbCAM) were tested by immunofluorescence. Briefly, cell slides were pre-cooled with 4% paraformaldehyde (PFA) (in PBS) and fixed at 4°C for 40 minutes. PFA was aspirated at room temperature and washed with a reagent (0.1% Triton X-100 in PBS). After washing with PBS, the corresponding primary antibody working solution (in 3% BSA in PBS) was added and incubated overnight at 4°C. After washing with PBS, the fluorescently labeled secondary antibody was added and incubated at room temperature in the dark (US AbCAM). The cells were stained with Hoechst in the dark for 10 min; then mounted in mounting medium and photographed.

Grouping of experiments

Placental trophoblast cells from 20 maternal women were randomly divided into 4 groups with 5 patients in each group. The cells were placed in a sterile perforated culture dish and set into a sterile closed box (the closed box was designed to be tightly connected to the anesthesia machine threaded tube circuit). The sterile closed box was placed in a 37°C water bath. Sevoflurane intervention was performed in 4 groups: 0 minute (S0), 15 minutes (S15), 30 minutes (S30) and 60 minutes (S60).

Sevoflurane intervention

Strictly doing the sterilization of the operating room and operating equipment to ensure that the entire intervention process was carried out under aseptic conditions. The anesthesia machine was started in a constant pressure self-test mode, the mixed gas was adjusted to mix air with 5% CO_2, and the 3% sevoflurane volatile tank was opened. When the anesthesia monitor showed that all gas concentrations reached the established standards, the anesthesia threaded tube was connected to the sterile closed box, and the placental trophoblast cells were treated with 3% sevoflurane according to different time conditions. After the intervention, the cells were cultured in an incubator with 5% CO_2 at 37°C for 24 hours.

Terminal-deoxynucleotidyl transferase mediated nick end labeling assay (TUNEL)

The apoptosis of placental trophoblast cells was observed by TUNEL assay. Briefly, cell slides were fixed with 4% PFA for 1 h at room temperature and then treated with 0.3% Triton X-100. After washing three times with PBS, the cells were covered with equilibration buffer. After equilibration, a 50 uL of rTdT incubation buffer was added on the cells and incubated at 37°C for 60 minutes in the dark wet box. The reaction was quenched with deionized water (1:10 dilution 20X SSC) at room temperature. The cell slides were immersed in fresh PBS to remove unincorporated fluorescein-12-deoxyuridine uridine. After that, the cells were stained with Hoechst in the dark for 10 min; then mounted in mounting medium and photographed. The area of the villous area (μm^2) and the number of apoptotic cells (number/10000 μm^2) were measured by an image analysis system, and the apoptosis rate (%) were calculated.

Enzyme linked immunosorbent assay (ELISA)

The concentrations of IL-6 and TNF-α in the cell culture medium was determined by ELISA. The process is as follows: (1) after setting the standard well and the sample hole, the total 100 μL of the standard sample and the sample were separately added to the respective wells, gently mixed by shaking, and incubated at 37°C for 90 minutes; (2) after discarding the liquid, a total of 100 μL of biotin-labeled antibody working solution was added to each well and incubated at 37°C for 60 minutes; (3) after discarding the liquid, a total of 100 μL horseradish peroxidase-labeled avidin working solution was added to each well and incubated at 37°C for 60 minutes; (4) after discarding the liquid, the plate was washed 5 times and dried, then a substrate solution of 90 μL was added to each well, and the color was developed at 37°C in dark room for 20 minutes; and (5) after adding 100 μL of the stop solution, the optical density of each well was measured with a microplate reader at a wavelength of 450 nm within 5 minutes.

Western blot

The total protein of placental trophoblast cells was extracted using RIPA lysate. The protein concentrations were quantified using Pierce™ BCA Protein Assay Kit (ThermoFisher, Waltham, USA). The proteins were transferred to the PVDF membrane by SDS-PAGE electrophoresis. After blocking with 5% skim milk, the corresponding primary antibody (IL-6 antibody,

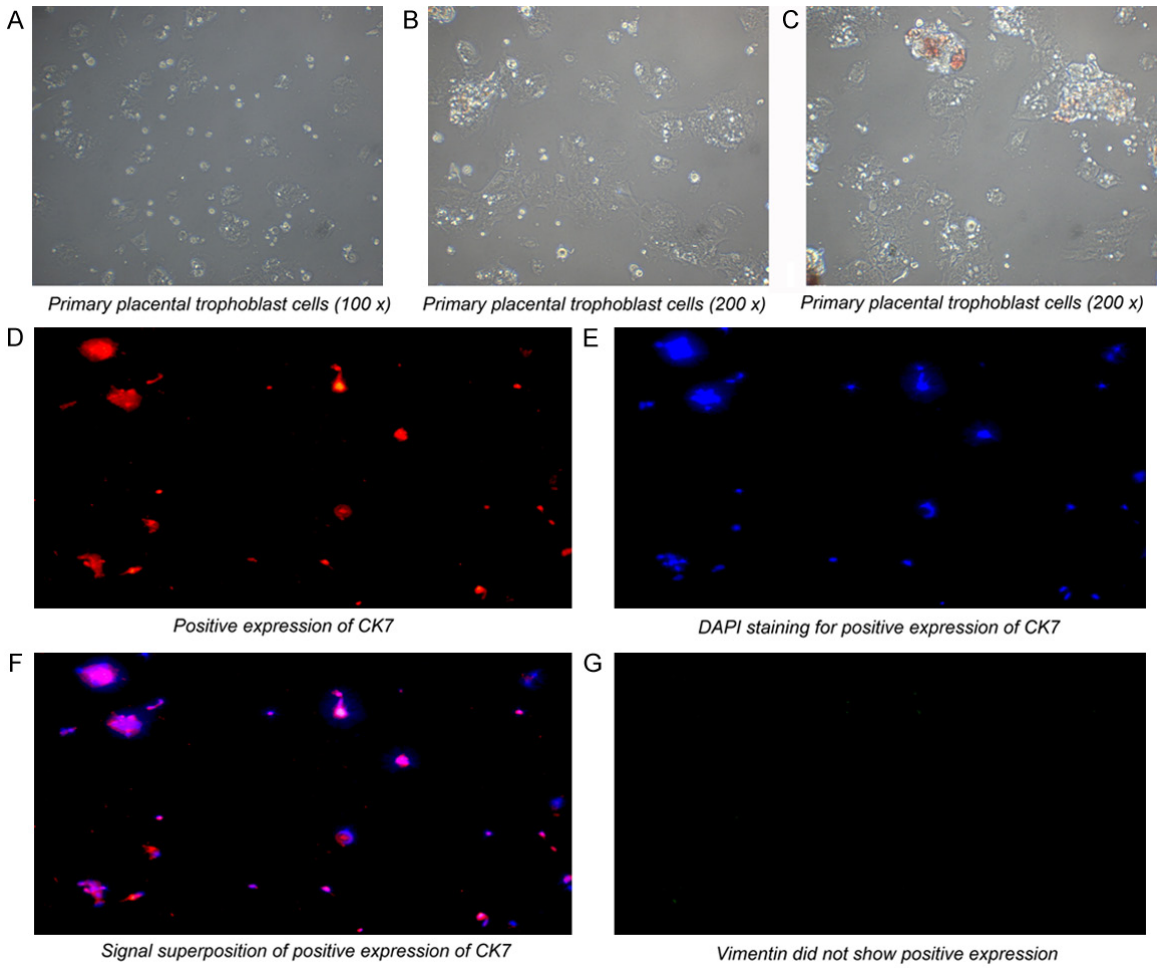

Figure 1. Cell morphology identification of isolated placental trophoblast cells. A. Most of the trophoblast cells fused and formed multinucleated syncytiotrophoblast cells (100×). B and C. Morphological map of placental primary trophoblast cells after 24 hours isolation (200×), the volume of cells were large in volume and the nucleus wwere oval, and riched in cytoplasm. D. Identification by CK7 immunofluorescence technique showed that CK7 was mainly expressed in the nucleus. E. DAPI staining suggested that CK7 was positively expressed in the nucleus of placental trophoblast cells. F. A superimposed image of DAPI staining and CK7 expression further confirmed that CK7 is mainly expressed in the nucleus of placental trophoblast cells. G. Identification by Vimentin immunofluorescence technique showed that the placental trophoblast cells did not show Vimentin expression. CK7, cytokeratin 7; DAPI, 4',6-diamidino-2-phenylindole.

ab233706, Abcam, USA; anti-TNF-a, ab183-218, Abcam, USA) was incubated at 4°C overnight. After washing the membrane twice with TBST for 7 minutes three times, the diluted secondary antibody (ab6721, Abcam, USA) was incubated for 1 h at room temperature. After washing with TBST, the membrane was tested by chemiluminescence method (ECL, Forevergen). Image J was used to analyze the optical density of the target strip. GAPDH (HC301, 1:5000) was used as an internal reference.

Statistical processing of data

All data were statistically processed using the SPSS 22.0 software package. The data belonged to the continuous variables was assessed by Student's t-test, Kruskal-Wallis test and One-way ANOVA. Measurement data were expressed as mean ± standard deviation. The Fisher's exact test and the one-way ANOVA were used for comparison of counting data between groups. The Student-Newman-Keuls method was used to compare the difference between the groups. Correlation analysis between the enumeration data was performed by the cruskal-wallis rank sum test. The descriptive statistics of the count data were expressed by rates and the chi-square test was used to compare the rates between the groups. $P<0.05$ was considered to be statistically significant.

Results

A good comparability was shown between the experimental groups

As shown in **Table 1**, according to the maternal placental trophoblast cells treated with sevoflurane at different times, the women were divided into 5 groups: 0 minute (S0), 15 minutes (S15), 30 minutes (S30) and 60 minutes (S60). Regardless of maternal age, gestational time, BMI, and the amount of ephedrine used, there was no statistical difference between the five groups (P>0.05). In addition, there was no difference in the incidence of scar uterus and fetal position abnormalities in the five groups of women (P>0.05).

Identification of primary trophoblast cells of placenta

The isolated primary placental trophoblast cells were cultured for 24 hours and observed under a fully automated erect fluorescence microscope Imager Z1 (Olympus USA). It can be seen that most of the trophoblast cells fused and formed multinucleated syncytiotrophoblast cells. The cell was large and the nucleus was oval, and riched in cytoplasm (**Figure 1A-C**). The CK7 was found to be positive and mainly expressed in the nucleus, but vimentin was negative, which indicates that the isolated primary cells were placental trophoblast cells with high purity (**Figure 1D-G**).

Sevoflurane induces apoptosis of isolated placental trophoblast cells

As shown in **Figure 2**, the nucleus of the apoptotic placental trophoblast cells showed a blue-violet color by fluorescence microscopy. The morphology of the cells was diverse, some were condensed into a mass and some were completely fragmented. The villus area did not show a difference between S0 (220637.79 μm^2), S15 (220701.57 μm^2), S30 (220422.34 μm^2) and S60 (218964.43 μm^2) groups (P>0.05) (**Table 2**; **Figure 3A**). However, the number and rate of apoptosis in the S30 (1.599±0.342/10000 μm^2; 3.19±1.01%) and S60 (3.0119±0.43/10000 μm^2; 4.77±2.55%) groups was higher than that in S0 (0.032±0.020/10000 μm^2; 0.78±0.59%) and S15 (0.049±0.025/10000 μm^2; 0.89±0.63%) groups (P<0.05) (**Table 2**; **Figure 3B** and **3C**). Moreover, the effect of sevoflurane on the apoptosis of placental trophoblast cells showed a time-dependent pattern.

Intervention of sevoflurane enhances secretion of TNF-α and IL-6 in placental trophoblast cells

The level of TNF-α in cell culture medium in S30 (17.21±6.71 pg/mL) and S60 (19.91±4.13 pg/mL) groups was significantly higher than that in S0 (3.36±1.29 pg/mL) and S15 (4.15±2.29 pg/mL) groups (P<0.05) (**Table 3**; **Figure 4A**). The level of IL-6 in S30 (6.27±2.31 pg/mL) and S60 (8.81±3.26 pg/mL) groups also showed a significant increase compared with S0 (3.82±1.42 pg/mL) and S15 (4.58±2.94 pg/mL) groups (P<0.05) (**Table 3**; **Figure 4B**). Moreover, the effect of sevoflurane on IL-6 and TNF-α showed a time-dependent pattern.

There is a positive correlation between expressions of TNF-α and IL-6 and apoptosis of placental trophoblast cells

As shown in **Figure 4C** and **4D**, the expressions of TNF-α and IL-6 and the apoptosis of cytotrophoblast cells showed a synchronous upward trend. The rank correlation coefficient was 0.735 and 0.806 respectively (P<0.5), according to the level of α equal to 0.05, the null hypothesis is rejected, so it can be considered that there is a positive correlation between the expressions of TNF-α and IL-6 and the apoptosis of placental trophoblast cells.

Intervention of sevoflurane increases expressions of TNF-α and IL-6 protein in placental trophoblast cells

As shown in **Figure 5A-C**, Western-blot showed that expressions of TNF-α and IL-6 in S30 and S60 groups were significantly higher than that in S0 and S15 groups (P<0.05). In addition, the expression of IL-6 in the S60 group was significantly higher than that in the S30 group (P<0.05).

Discussion

The American Obstetric Anesthesia Guidelines published in 2017 indicate that spinal anesthesia is preferred for cesarean section anesthesia, however, spinal anesthesia also has certain contraindications for obstetric applications

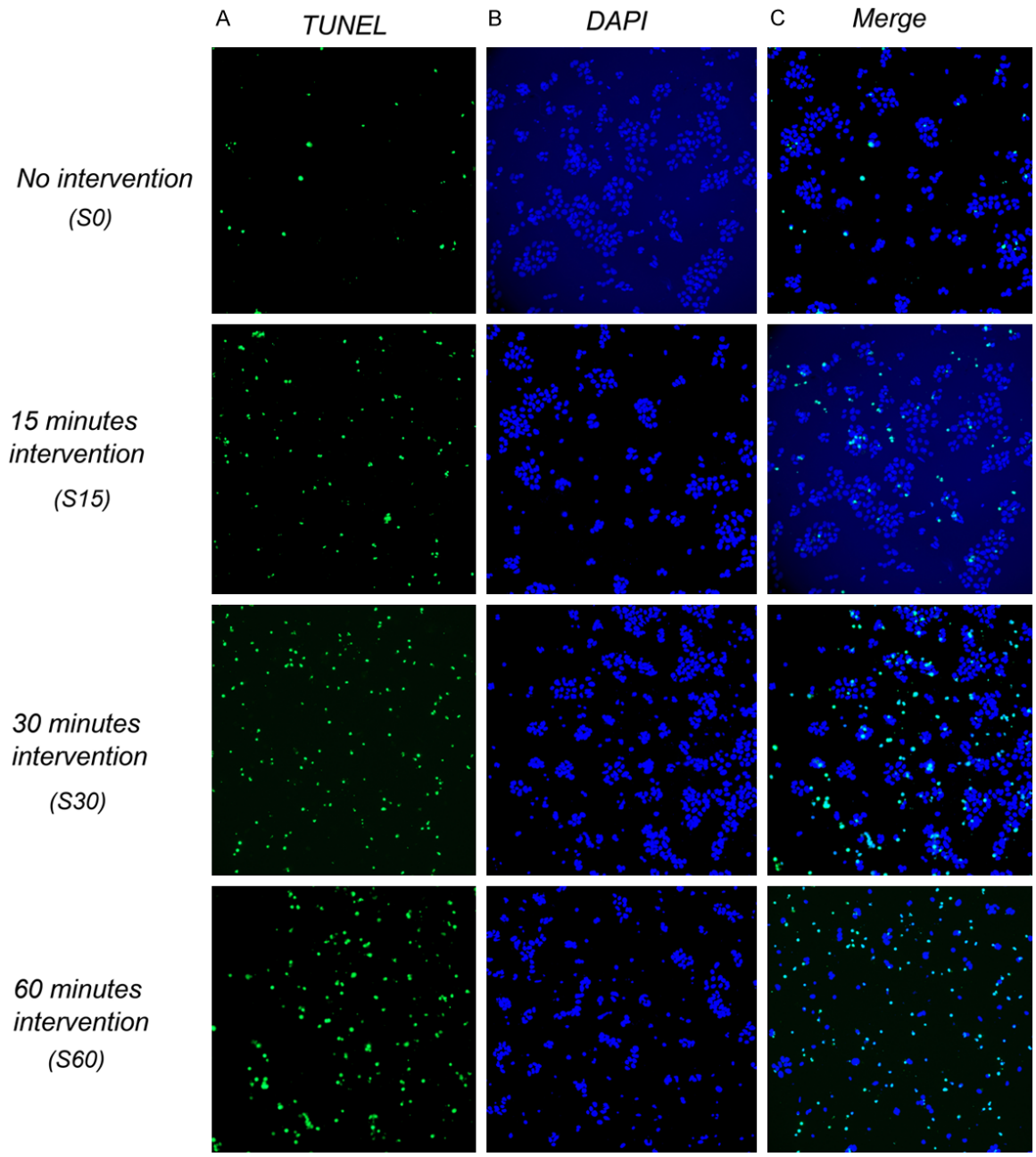

Figure 2. Detection of apoptosis of primary trophoblast cells by TUNEL assay. A. The nucleus of apoptotic cells showed green fluorescence and their morphology was different. Some of them were condensed into clusters and some were completely fragmented. TUNEL assay showed that apoptotic cells in the S30 and S60 groups were increased obviously compared to the S15 and S0 groups. B. DAPI staining showed that the nucleus of placental trophoblast cells was blue-violet. Compared with the S15 and S0 groups, the number of cells in the S30 and S60 groups was significantly reduced. C. A superimposed image further confirmed that as the sevoflurane intervention time prolonged, the apoptosis of placental trophoblast cells gradually increased. TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling assay; DAPI, 4',6-diamidino-2-phenylindole.

[9]. In China, to maternal complications with pathological disease or contraindications for spinal anesthesia, general anesthesia is often used in cesarean section, or supplemented with general anesthesia. Because the development of fetal organs is still imperfect, the specific effects of anesthetic drugs transported through the mother's placenta to the fetus are not clear [10]. Fetal delivery is often accompanied by a certain degree of hypoxic emergency,

Table 2. Comparison of villus area, apoptotic number and apoptotic rate in each group (X±S)

Groups	N	Villus area (μm²)	*P* value	Number of apoptosis (Per 10000 μm²)	*P* value	Apoptotic rate (%)	*P* value
Comparison between different intervention time groups and non-intervention group							
S0	5	220637.79	>0.05	0.032±0.020	>0.05	0.78±0.59	>0.05
S15	5	220701.57		0.049±0.025		0.89±0.63	
S0	5	220637.79	>0.05	0.032±0.020	<0.05	0.78±0.59	<0.05
S30	5	220422.34		1.599±0.342		3.19±1.01	
S0	5	220637.79	>0.05	0.032±0.020	<0.05	0.78±0.59	<0.05
S60	5	218964.43		3.0119±0.43		4.77±2.55	
Comparison between different intervention time groups							
S15	5	220701.57	>0.05	0.049±0.025	<0.05	0.89±0.63	<0.05
S30	5	220422.34		1.599±0.342		3.19±1.01	
S15	5	220701.57	>0.05	0.049±0.025	<0.05	0.89±0.63	<0.05
S60	5	218964.43		3.0119±0.43		4.77±2.55	
S30	5	220422.34	>0.05	1.599±0.342	<0.05	3.19±1.01	<0.05
S60	5	218964.43		3.0119±0.43		4.77±2.55	

X±S, mean ± standard deviation.

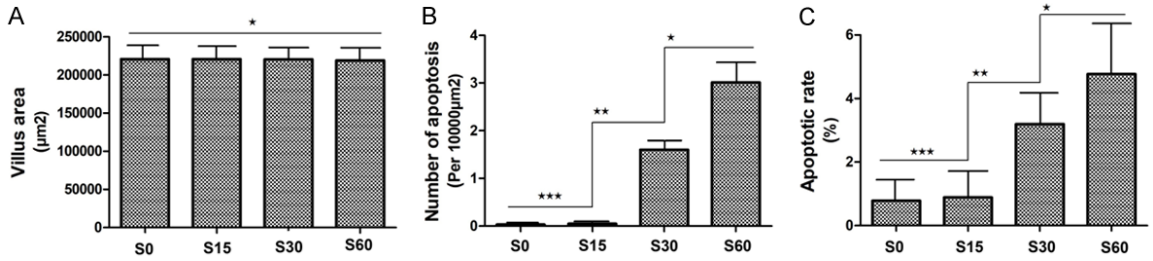

Figure 3. Effect of sevoflurane intervention on apoptosis of placental trophoblast cells at different times. A. *There was no statistically significant difference on the area of villus between the intervention groups (P>0.05). B. *The number of apoptotic cells in S60 group was higher than that in S30, S15 and S0 groups (P<0.05); **the number of apoptotic cells in S30 group was higher than that in S15 and S0 groups (P<0.05); ***there was no significant difference between S15 and S0 groups (P>0.05). C. *The apoptotic rate in S60 group was higher than that in S30, S15 and S0 groups; **the apoptotic rate in S30 group was higher than that in S15 and S0 groups; ***there was no significant difference on the apoptotic rate between S15 and S0 groups (P>0.05). S0, sevoflurane intervention of 0 minute, S15, sevoflurane intervention of 15 minutes, S30, sevoflurane intervention of 30 minutes, S60, sevoflurane intervention of 60 minutes.

while fetuses in hypoxic stress will produce a large number of free radicals during reperfusion, which may lead to ischemia-reperfusion injury [11]. Currently, some volatile anesthetics are often used in general anesthesia, and these drugs themselves may cause the formation of free radicals and affect the mother and the fetus. However, different volatile anesthetics may cause different levels of free radical production in the body [12]. The mechanism of action of sevoflurane in maternal and fetal bodies is unclear. Although sevoflurane increases the formation of free radicals, it can play a role in pre-treating and protecting cells from ischemia-reperfusion injury [13, 14].

The placenta is an important part of the direct contact between the mother and the fetus, and is also an important organ for material exchange between the mother and the fetus. In order to reveal the effects of sevoflurane on maternal placental trophoblast cell apoptosis and proinflammatory cytokine secretion, we used different time gradients of 3% sevoflurane to intervene in placental trophoblast cells in vitro. We divided the experiments into 5 groups according to the different time of sevoflurane intervention in placental trophoblast cells, namely 0 minute (S0), 15 minutes (S15), 30 minutes (S30) and 60 minutes (S60). We found that there was no difference on villus area was

Table 3. Comparison of IL-6 and TNF-α concentrations in placental trophoblast cell culture medium under different intervention time of sevoflurane (X±S)

Comparison of IL-6 and TNF-α concentrations between different intervention time groups and non-intervention group					
Groups	N	TNF-α (pg/mL)	*P* value	IL-6 (pg/mL)	*P* value
S0	5	3.36±1.29	>0.05	3.82±1.42	>0.05
S15	5	4.15±2.29		4.58±2.94	
S0	5	3.36±1.29	<0.05	3.82±1.42	<0.05
S30	5	17.21±6.71		6.27±2.31	
S0	5	3.36±1.29	<0.05	3.82±1.42	<0.05
S60	5	19.91±4.13		8.81±3.26	
Comparison of IL-6 and TNF-α concentrations between different intervention time groups					
S15	5	4.15±2.29	<0.05	4.58±2.94	<0.05
S30	5	17.21±6.71		6.27±2.31	
S15	5	4.15±2.29	<0.05	4.58±2.94	<0.05
S60	5	19.91±4.13		8.81±3.26	
S30	5	17.21±6.71	>0.05	6.27±2.31	<0.05
S60	5	19.91±4.13		8.81±3.26	

X±S, mean ± standard deviation; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

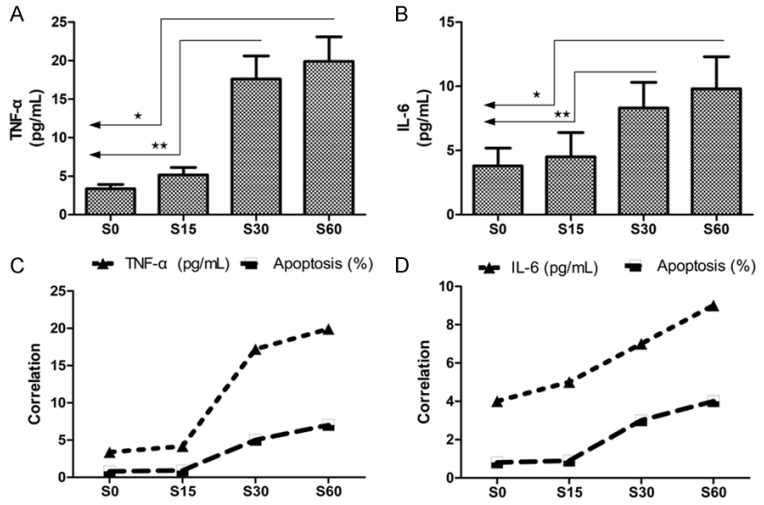

Figure 4. Comparison of TNF-α and IL-6 concentrations in the culture medium of placental trophoblast cells. A. *The concentration of TNF-α in S60 group was higher than that in S15 and S0 groups (P<0.05); **the concentration of TNF-α in S30 group was higher than that in S15 and S0 groups (P<0.05). B. *The concentration of IL-6 in S60 group was higher than that in S15 and S0 groups (P<0.05); **the concentration of IL-6 in S30 group was higher than that in S15 and S0 groups (P<0.05). C. The expression of TNF-α is positively correlated with the apoptosis of placental trophoblast cells (P<0.05). D. The expression of IL-6 is positively correlated with the apoptosis of placental trophoblast cells (P<0.05). TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; S0, sevoflurane intervention of 0 minute, S15, sevoflurane intervention of 15 minutes, S30, sevoflurane intervention of 30 minutes, S60, sevoflurane intervention of 60 minutes.

observed between different time groups. However, after more than 30 minutes of 3% sevoflurane intervention in placental trophoblast cells, the number and rate of cell apoptosis per 10000 μm^2 of villus area were significantly higher than that of non-intervention and 15 minutes intervention groups, which means that prolonged use of sevoflurane promotes the apoptosis in placental trophoblast cells. Apoptosis is the active death process of cells, and excessive apoptosis affects the integrity of organ structure and function. It is suggested that apoptosis is not only a special type of cell death, but also has important biological significance and complex molecular biological mechanisms [15]. Today, the effects of inhaled anesthetics such as isoflurane and sevoflurane on apoptosis are still controversial. It is reported that inhaled anesthetics can induce lymphocyte apoptosis, and the incidence of apoptosis is related to dose and time [16], especially inhalation anesthesia with isoflurane can cause brain cell apoptosis during fetal development [17, 18]. Pregnancy is a process in which mothers interact with the fetus and coexist with each other. The integrity of the placental function and structure is an important prerequisite for hormone secretion, immune regulation and substance exchange. Through the observation of apoptosis in placental trophoblast cells in our study, we considered that the use of sevoflurane in maternal anesthesia should not be too long, preferably within 15 minutes, which has the least effect on the apoptosis of placental trophoblast cells.

Apoptosis also has some self-regulatory mechanisms. Some pro-inflammatory factors can

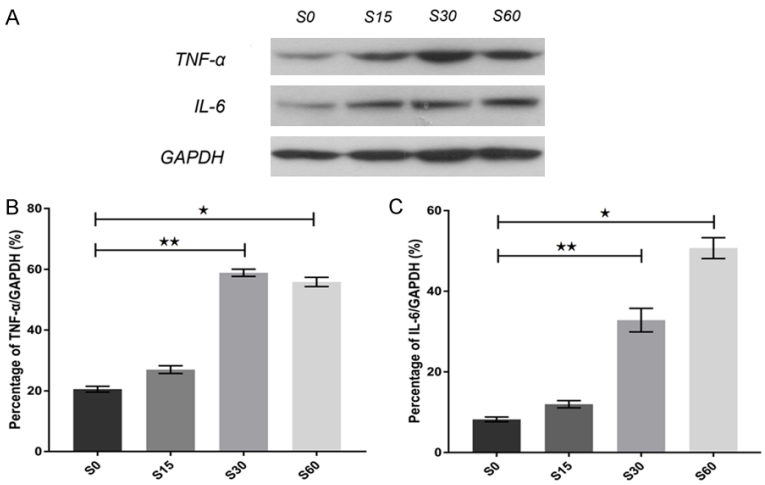

Figure 5. Expression levels of TNF-α and IL-6 proteins in placental trophoblast cells. A. Western blot showed that TNF-α and IL-6 were significantly elevated in the S30 and S60 groups compared with the S15 and S0 groups. B. Gray fog detection indicated that ★the expression of TNF-α in S60 group was higher than that in S15 and S0 groups and that ★the expression of TNF-α in S30 group was higher than that in S15 and S0 groups ($P<0.05$). C. Gray fog detection indicated that ★the expression of IL-6 in S60 group was higher than that in S30, S15 and S0 groups and that ★the expression of IL-6 in S30 group was higher than that in S15 and S0 groups ($P<0.05$). TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; GADPH, glyceraldehyde-3-phosphate dehydrogenase; S0, sevoflurane intervention of 0 minute, S15, sevoflurane intervention of 15 minutes, S30, sevoflurane intervention of 30 minutes, S60, sevoflurane intervention of 60 minutes.

induce apoptosis, and others inhibit apoptosis [19]. The apoptosis of placental trophoblast cells is greatly affected by internal and external environment factors. In our study, we found that with the prolongation of sevoflurane intervention, the expressions of TNF-α and IL-6 in placental trophoblast cell culture medium and cells itself increased significantly, which means that sevoflurane stimulates placental trophoblast cells to secrete TNF-α and IL-6. A study suggests that TNF-α can induce apoptosis in syncytiotrophoblasts and cytotrophoblasts, which may be achieved by increasing the levels of nitric oxide or reactive oxygen species [20]. The apoptotic trophoblast cells form syncytiotrophoblast derived microvesicles (STBM), and STBM can stimulate monocytes and natural killer cells to secrete some pro-inflammatory factors including TNF-α, IL-6, IL-8 and so on [21]. The results we observed were that when sevoflurane was administered for more than 30 minutes, the expressions of TNF-α and IL-6 in trophoblast cells increased, and the apoptosis number of trophoblast cells also increased. The short-term (within 15 minutes) use of sevoflurane did not cause apoptosis in placental trophoblast cells and an increase in pro-inflammatory factors. Therefore, our results suggest that sevoflurane should not be used for more than 30 minutes when anesthetizing pregnant women. The purpose is to minimize the effect of sevoflurane on the function of placental trophoblast cells, so that the growth and development of the fetus in the mother is not affected by the drug.

At present, there are few studies on the effects of anesthetics on placental trophoblast cells, its influence on the apoptosis of placental trophoblast cells and its regulation mechanism are still unclear. Our study only explored the apoptosis of placental trophoblast cells and the changes of inflammatory factor expression levels in cells under the intervention of 3% sevoflurane. Although we conclude that sevoflurane-induced apoptosis in placental trophoblast cells may be associated with the phenomenon that it promotes the expressions of TNF-α and IL-6, its specific intrinsic mechanisms still require in-depth research in future. However, the study expands on new ideas for the safe use of narcotic drugs in pregnancy.

Conclusion

Intervention of maternal-differentiated placental trophoblast cells with 3% sevoflurane for more than 30 minutes promotes trophoblast cell apoptosis and increases TNF-α and IL-6 secretion, suggesting that the use time of sevoflurane in maternal induction anesthesia should be preferably within 15 minutes to reduce the effect of this drug on the function of placental trophoblast cells.

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Disclosure of conflict of interest

None.

Abbreviations

CK7, cytokeratin 7; DMEM, dulbecco's minimum essential medium; ELISA, enzyme-linked immunosorbent assay; FDA, Food and Drug Administration; PBS, phosphate buffered saline; PFA, paraformaldehyde; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling assay.

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