Original Article
Neurodevelopmental outcomes after a brief exposure to the inhaled anesthetic sevoflurane in young children undergoing palatoplasty

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Abstract: Objective: The purpose of this study was to investigate whether a brief exposure to sevoflurane can lead to adverse neurodevelopmental outcomes in children undergoing palatoplasty. Materials and methods: Young children (less than 2 years old) receiving palatoplasty with sevoflurane anesthesia were recruited. The Bayley Scales of Infant Development-Second Edition (BSID-II) assessment was used to evaluate neurodevelopmental outcomes before and after surgery. The levels of plasma neuron-specific enolase and S-100β were also measured. Results: This study enrolled 101 patients and 100 healthy children for analysis. The sevoflurane exposure time of all patients was 46.2±6.5 minutes. There were no statistically significant differences noted between preoperative and postoperative BSID-II scores, plasma neuron-specific enolase and S-100β values. When compared with normal healthy children, there were also no statistically significant differences between patients and normal healthy children at the age of 3-4 years in the BSID-II scores ($P>0.05$). Conclusions: The data did not show any significant evidence of an association between a brief exposure to sevoflurane during early childhood and adverse neurodevelopmental outcomes in children undergoing palatoplasty.

Keywords: Anesthesia, sevoflurane, cleft palate, children, neurodevelopment

Introduction

General anesthetics and sedative drugs are administered to millions of infants, toddlers and preschool children each year [1]. However, substantial data from animal studies have shown that general anesthetics may cause neurotoxic changes in the developing brain that leads to adverse neurodevelopmental outcomes later in life [2-4]. As a result, neurodevelopmental safety concerns about the use of anesthesia during early development have arisen. Cleft palate is one of the most common congenital facial anomalies that require surgical repair in the early age. So most cleft palate patients received palatoplasty at 9-12 months of age to improve early speech outcomes [5]. During this period, the immature brain is in the stage known as the "brain's growth spurt" (BGS), which begins at mid-gestation and continues for 2 to 3 years after birth [6, 7]. The

immature brain is most vulnerable to neurotoxic agents during the BGS period [8]. Sevoflurane is one of the most frequently used volatile anesthetics for the induction and maintenance of general anesthesia during surgery because of its low blood-gas partition coefficient and low pungency. In infants and children, these properties convey the benefit of rapid induction and recovery and reduce irritation to the airway [9]. Several recent animal studies have shown that exposure to sevoflurane may have neurotoxic effects on the immature brain, and these effects can lead to long-term cognitive impairment [4, 9-16]. However, animal models may not accurately represent the pathophysiological processes in humans because of known interspecies variability [17]. Though several retrospective cohort studies focusing on anesthetic-induced neurotoxicity in young children were of great significance, they were lacking of persuasion and did

not reflect the patient characteristic and cultural and racial/ethnic diversity of the overall population [18-20]. For lack of detailed anesthetic information and medical records, we did not know the exact anesthetic agents used in these retrospective cohort studies and the relationship between sevoflurane exposure and neurotoxic changes during early childhood.

The Bayley Scales of Infant Development-Second Edition (BSID-II) is successfully used to predict neurodevelopmental outcomes after surgery in infants and is suitable for infants between 1 and 42 months of age [21, 22]. The mental and motor scales of the BSID-II yield standardized scores; these are the Mental Development Index (MDI) and the Psychomotor Development Index (PDI), respectively. In addition, specific brain-originated proteins, such as neuron-specific enolase (NSE) and S-100β, are accepted as independent predictors of poor neuropsychological outcomes after surgery [21, 23]. In this study, we investigated the neurodevelopmental outcomes after a brief sevoflurane exposure in young children undergoing palatoplasty using the BSID-II assessment and biomarkers of poor neuropsychological outcomes as previously described [21].

Patients and methods

This prospective study was approved by the Ethics Committee of Shanghai Ninth People's Hospital, affiliated to the Shanghai Jiao Tong University School of Medicine (Shanghai, China). Informed consent and approval were obtained from the parents prior to the study. The study was conducted in accordance with the Declaration of Helsinki. Young children (less than 2 years old) with American Society of Anesthesiologists status I, scheduled for palatoplasty were enrolled into this study over an 18-month period. This type of operation requires less than 1 hour in our hospital. Cleft palate can be part of many syndromes, including Pierre Robin, Treacher-Collin's, or Goldenhar, and children with these syndromes were excluded [24]. Children born prematurely and those with a history of rescue at birth, cerebral anoxia, malnutrition, previous surgery/anesthesia, trauma history, or other diseases, such as pneumonia or meningitis, were also excluded.

The anesthesia procedure used in this study is our routinely used protocol which is similar to previously described method [24, 25]. Specifically, parents were asked to refrain from feeding their children for 6 hours prior to surgery. The children did not receive any premedication. On the day of surgery, the children were transferred to the operating theatre and pulse oximetry, ECG and non-invasive arterial pressure were monitored. The children received 8% sevoflurane (Baxter®) with 100% oxygen at a flow of 6 liters min^{-1} for anesthesia induction using an air cushion face mask of appropriate size and an inhalational anesthesia circle system (Datex Ohmeda (S/5 Avance) anesthesia machine with Drager Vapor 2000). After the children were quiet and lost eyelash reflex, an intravenous cannulation was inserted into a peripheral vein for fluid infusion. Then, muscle relaxant rocuronium (0.5 mg·kg^{-1}) was injected before intubation. The sevoflurane was maintained at 8% until laryngoscopy. The face mask ventilation was assisted using a 10-15 cmH$_2$O inspiratory pressure at a ventilatory frequency of 18-20 min^{-1}. The trachea was orally intubated 150 s after induction via a direct laryngoscopy by a senior pediatric anesthetist. After intubation, the concentration of the inhaled sevoflurane was reduced to 2-3% to maintain anesthesia during the operation. The cleft palate surgeon was allowed to use 1% lidocaine 5-10 ml for local infiltration anesthesia. The volume of ventilation was 10 ml·kg^{-1} at a ventilator frequency of 18-22 min^{-1}, which maintained the exhaled concentrations of carbon dioxide at 35-45 mmHg. The sevoflurane exposure time (from induction to completion of the operation) was recorded. The heart rate, pulse oximetry, and the non-invasive blood pressure were also recorded before anesthesia induction (baseline value) and then every 5 min after anesthesia induction. Hemodynamic adverse events were defined as bradycardia, tachycardia, hypertension, or hypotension (variation ≥30% from baseline value). The anesthetist maintained the blood pressure and heart rate within 30% of baseline values by adjusting the sevoflurane concentration. The respiratory adverse events were defined as bronchospasm or oxygen saturation <95% during anesthesia. Patients with hemodynamic or respiratory adverse events were also excluded from this study. After surgery, the patients were transferred to a post-

Table 1. Patient Characteristics before Anesthesia (n=101)

Age (months)	Weight (kg)	Females (%)	Heart rate (beats min⁻¹)	Mean blood pressure (mmHg)	Pulse oximetry (%)
12.9±1.7 (10~18)	10.7±1.5 (8.5~15.3)	41.1	126.9±8.4 (107~148)	59.7±4.9 (52~73)	99.5±0.5 (98~100)

Data are the mean ± standard deviation (range) for age, weight, heart rate, mean blood pressure and pulse oximetry.
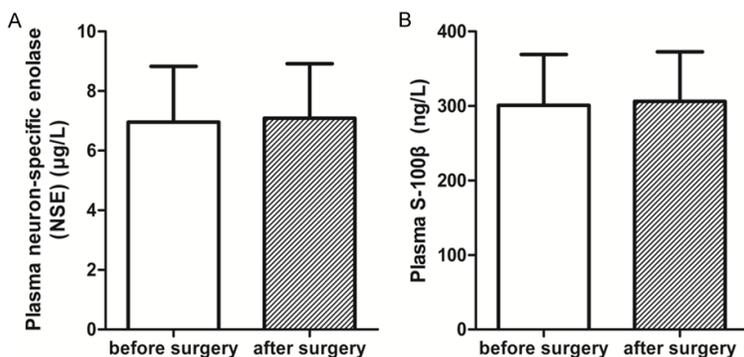
Figure 1. Mean plasma levels of the central nervous system injury biomarkers neuron-specific enolase (NSE) (A) and S-100β (B) before and after surgery (n=101).

anesthesia care unit, and the tracheal tube was removed when spontaneous breathing recovered. The children were observed in the post-anesthesia care unit for an additional 1 hour after extubation before being transferred to the ward.

The patients were assessed using the BSID-II on the day before surgery, 6 months and 18 months after the operation (when the children returned for postoperative follow-up) by an experienced pediatrician to determine the MDI score and PDI score. The Bayley assessments were normalized to the respective era. In order to compare the patients with normal healthy children, we also assessed the MDI sores and PDI sores using the BSID-II for 100 healthy children at 3 to 4 years of age in the community of Shanghai. To minimize child discomfort, 2 ml additional blood sample was obtained during a routine preoperative blood examination performed 1 day before surgery. The maximum expression of plasma biomarkers of poor neuropsychological outcomes may occur at 6 hours after surgery [21]. Thus, the second blood sample was obtained 6 hours after the operation. Blood plasma was removed after centrifugation and stored at -80°C for later analysis of NSE and S-100β. Enzyme-linked immunosorbent assay-based tests were used to measure the plasma NSE (NSE ELISA

Kit, antibodies-online, Germany) and S-100β (S-100β EL-ISA Kit, antibodies-online, Germany) levels. For ethical considerations, we didn't obtain blood samples from normal heathy children.

Statistical analysis

Data is expressed as the mean ± standard deviation (SD). Statistical difference was determined using SAS statistics software (SAS System for Windows, Version 9.2; SAS Institute Inc, Cary, North Carolina). The paired samples *t*-test and *t*-test were used to investigate the differences in the MDI and PDI scores and NSE and S-100β values. The acceptance level for statistical significance was considered *P*<0.05.

Results

Among the 116 patients recruited in the study, 11 children were excluded owning to hemodynamic or/and respiratory adverse events. In the remaining 105 cases, 4 cases and 15 cases were excluded respectively during the 6 months and 18 months periods because of loss to follow-up. As a result, 101 patients with a 6 months follow-up and 86 patients with an 18 months follow-up were analyzed.

The characteristic data of the subjects is given in **Table 1**. The sevoflurane exposure time of all patients was 46.2±6.5 (range 31-58) minutes. All of the 101 patients received 98-100% pulse oximetry, and no adverse events occurred during anesthesia. The mean postoperative plasma NSE value was higher than before surgery (before vs after; µg/L): 6.96 ±1.87 vs 7.09±1.83 (**Figure 1A**). However, there was no statistically significant difference in the pre- and postoperative NSE values (*P*= 0.1138). Similarly, the mean S-100β value 6 hours after surgery was slightly higher than be-
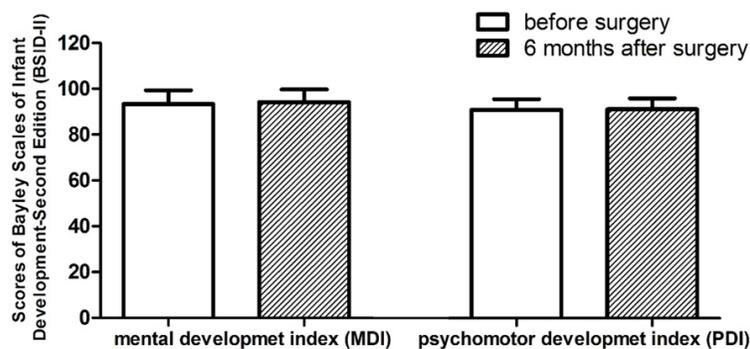
Figure 2. The mental development index (MDI) and psychomotor development index (PDI) from Bayley Scales of Infant Development-Second Edition (BSID-II) evaluation before and 6 months after surgery (n=101).
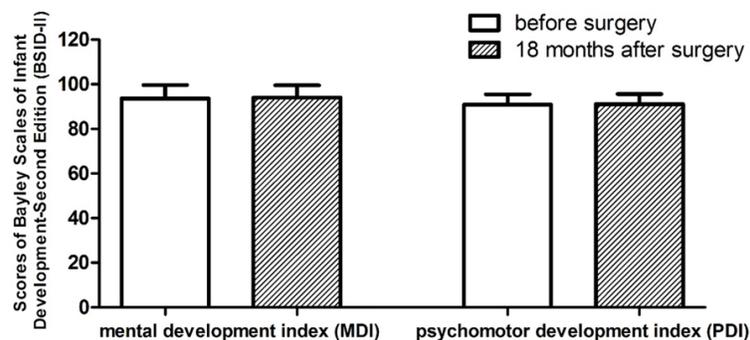
Figure 3. The mental development index (MDI) and psychomotor development index (PDI) from Bayley Scales of Infant Development-Second Edition (BSID-II) evaluation before and 18 months after surgery (n=86).

fore surgery (before vs after; ng/L): 300.8± 68.3 vs 306.1±66.5 (**Figure 1B**), but the difference was not statistically significant (*P*= 0.1956).

For the 6 months follow-up patients, there were no statistically significant differences between the pre- and 6 months postoperative MDI sores of BSID-II assessment (*P*=0.1103) (**Figure 2**). There was a slight improvement in the MDI score 6 months after surgery (before vs after): 93.6±5.9 vs 94.1±5.5 (**Figure 2**). A similar result was obtained for the PDI scores (before vs after): 90.0±4.8 vs 91.2±4.7 (**Figure 2**). There were no significant changes in PDI scores pre- and 6 months post-operative (*P*=0.2911).

For the 18 months follow-up patients, there were also no statistically significant differences between the pre- and 18 months postoperative MDI sores of BSID-II assessment (*P*= 0.1087) (**Figure 3**). There was a slight improve-

ment in the MDI score 18 months after surgery (before vs after): 93.7±6.1 vs 94.1± 5.4 (**Figure 3**). A similar result was obtained for the PDI scores (before vs after): 90.8± 4.8 vs 91.1±4.4 (**Figure 3**). There were also no significant changes in PDI scores pre- and 18 months post-operative (*P*=0.1133).

When compared with normal healthy children, there were also no statistically significant differences between postoperative patients and normal healthy children the age of 3-4 years in the MDI scores (patients vs health): 94.1±5.4 vs 93.4±4.7, (*P*=0.3458) and PDI scores 91.1±4.4 vs 92.5±6.2, (*P*=0.0820).

Discussion

In this study, we examined whether the widely used inhaled anesthetic sevoflurane is neurotoxic to young children undergoing palatoplasty and found the result was not overtly suggestive of neurotoxic effects following a brief exposure to sevoflurane on the developing brain. Though this result was negative, it was important to the clinical practice. Recently, growing data from animal studies raised the possibility that early exposure to anesthetics could have a negative impact on the neurodevelopment of young children, which is of concern for both parents and anesthetists. However, anesthesia researcher and regulators at the Food and Drug Administration are reluctant to make recommendations to parents and physicians based on these animal data alone, so large-scale clinical studies are urgently needed. In this analysis, we chose NSE and S-100β as independent predictors of poor neuropsychological outcomes, because the elevations of these proteins in blood were confirmed to have a close relationship with central nervous system injury [21]. We did not find significant increases in plasma NSE and S-100β after about 1 hour sevoflurane anesthesia compared to pre-anes-

thetic levels in this trial. This may indicate that no noticeable central nervous system damage occurs after a brief sevoflurane administration.

Though there are several retrospective cohort studies assessing the effect of anesthesia in infancy on long-term neurodevelopmental outcomes [20, 26, 27], few of these studies could establish a clear link between early anesthesia exposure and later neurodevelopmental anomalies because of the confounding factors. For lack of detailed anesthetic information and medical records, we did not know the exact anesthetic agents used in these retrospective cohort studies and the relationship between a simple sevoflurane exposure and neurotoxic changes during early childhood. Due to the use of balanced general anesthesia (volatile anesthetic used with other intravenous anesthetics) in routine surgical procedures, it is challenging to study the neurodevelopmental outcomes after an individual volatile anesthetic administration. In our study, we enrolled infants with cleft palate as this disease is one of the most common congenital facial anomalies and considered to be no association with intellectual development. Using lidocaine for local infiltration anesthesia and sevoflurane for anesthesia induction and maintenance, none other general anesthetics and sedative drugs, such as ketamine, propofol, etomidate, opioids and benzodiazepines, were needed in such operation. All these drugs mentioned above were proved to have correlation with neurodevelopmental anomalies in previous animal studies [28-31]. An internal audit of anesthetic duration in infants at Boston Children's Hospital showed that 53% of anesthetics done in babies younger than 12 months of age were less than 2 h in duration. Thus, palatoplasty may represent the majority of pediatric surgeries and provide appropriate duration of sevoflurane exposure.

When assessing cognition in children with early exposure to anesthesia, results may depend on the type of outcome measure used [32]. Although the published literature has presented contradictory conclusions, this may be due to the variability in the outcome measures used. In human cohorts, some researchers have found an association with a single brief exposure [33], whereas others have only found an association after longer or multiple expo-

sures [18]. The evaluation scales used for these studies may in part cause the conflicting results. BSID-II is widely used in early diagnosis of neurological impairment and developmental disorders in children. We used 2 components of the BSID-II: the Psychomotor Developmental Index (PDI), which assesses gross motor and fine motor skills, and the Mental Developmental Index (MDI), which measures cognitive functioning through assessment of memory, problem solving, number concepts, vocalization, and language and social interaction skills. The mean PDI and MDI score for the normal population is 100, with a SD of 15, and a minimum score of 50. Subjects who were too impaired to complete neurodevelopmental testing were assigned a score of 50. In our study, young patients were scheduled to visit the surgeon 6 months and 18 months after surgery and the BSID-II assessment was conducted again at those times. Some social or geographical factor might make the follow-up difficult (eg, some patients lived in remote mountainous areas and some patients' contact information changed). Through data analysis, we found no significant differences in MDI and PDI scores between pre-anesthesia and post-anesthesia. When compared to healthy children with the same age of 3 to 4 years, we also found no significant differences in MDI and PDI scores. The result indicated that, even compared with healthy children, exposure of just less than 1 hour to a sevoflurane general anesthesia in infancy may not increase the risk of adverse neurodevelopmental outcomes over a 18-month follow-up period. Coincidentally, our finding was similar to the secondary outcome of General Anesthesia compared to Spinal anesthesia (GAS) study (one of the two largest international multi-site randomized controlled ongoing trials) [34]. In the GAS study, the researchers had done a lot of work and found general anesthesia based on sevoflurane may not cause an adverse neurodevelopmental outcome at 2 years of age in infants who were anesthetized for inguinal herniorrhaphy. As this is multi-site randomized controlled study carried out in different countries, cultural and racial diversity of the overall population, anesthesia practice and the type and dose of local anesthetics used in surgery may be different and could cause some bias of the result. Only one evaluation of Bayley Scales was conducted after surgery (not

before surgery) in their study. In our study, we allowed each patient to serve as their own control, which could reduce individual differences to the maximum extent. Furthermore, all the patients received 98-100% pulse oximetry during surgery, and children with adverse events were excluded. Therefore, the influence of adverse events and the possibility of hypoxic brain injury may be eliminated from our analysis. Though we ruled out general anesthetics and sedative drugs, which may cause neurodevelopmental anomalies as many as possible and sevoflurane was the unique anesthetic agent used for this study, it is impossible to eliminate all confounding factors (especially surgery) that can affect the analysis on the results. It has also been reported that sevoflurane could induce emergence agitation in children after surgery [35, 36], the duration of emergence agitation is transient and the relationship between emergence agitation and neurodevelopment is unknown. Therefore, sevoflurane-induced emergence agitation is beyond the scope of our study.

Due to the difficulty with the study regarding anesthesia-induced neurodevelopmental outcomes in young children, there are several limitations of our work. First, we could not fully exclude confounders (especially the surgery) that may affect the results. As described above, providing anesthesia without surgery to young children is infeasible. As a result, though the plasma NSE and S-100β values were slightly higher after surgery, it is uncertain whether anesthesia or the surgery would be responsible for the results. Furthermore, for ethical considerations, we did not monitor continuous NSE and S-100β values after surgery because most parents are unwilling to have more additional blood samples collected from their children. We also didn't obtain blood samples from normal healthy children so we could not compare the patients with the healthy children for the plasma NSE and S-100β values. Second, many children with cleft palate also have a speech disturbance. As a result, the language testing of BSID-II was coarser and may have influenced the cognitive score. Fortunately, language testing is not abundant in BSID-II assessment of children less than 2 years old. Third, the assessment of BSID-II is suitable for young children between 1 and 42 months of age. As children get older, the assessment is not appropriate to detect the later neurobehavioral changes. Thus, we did not assess the cognitive outcomes when these children got older. Lastly, we only studied the neurodevelopmental outcomes of young children with a single and brief exposure to sevoflurane. As a result, outcomes caused by prolonged or repeated exposure to sevoflurane have not been examined. In our next plan, we will enroll patients with both cleft lip and palate and alveolar cleft for research. Whether multiple exposures to sevoflurane can cause neurodevelopmental impairment will be measured, as these patients need repeated surgery and anesthesia.

In conclusion, in this study of infants who underwent palatoplasty for cleft palate, we found no evidence of a significant association between a brief exposure to inhaled anesthetic sevoflurane and adverse neurodevelopmental outcomes. Thus, we suggest that there is insufficient clinical evidence for recommendation for changes in this type of pediatric practice. However, whether prolonged or repeated exposure to sevoflurane is clinically harmful to the developing brain is unclear. Anesthesiologists suggest that additional compelling evidence is needed to assess the neurodevelopmental risks of anesthesia exposure [37]. We expect more evidence to be obtained from well-designed prospective studies to guide our clinical practice so that we can provide the safest care for the most vulnerable patients.

Disclosure of conflict of interest

None.

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