Original Article

Impact of sevoflurane anesthesia on S-adenosylmethionine in neonates under general anesthesia

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Abstract

Background and Aims: Preclinical studies in rodents and primates have shown that anesthesia was neurotoxic to the developing brain after exposure in the neonatal period. Sevoflurane a commonly used inhalational anesthetic, especially in pediatric surgery, might cause behavioral impairment in the developing brain. Although favored for its rapid onset and minimal airway disturbance, sevoflurane has been implicated in neurotoxic effects such as anesthesia-induced developmental neurotoxicity in rodents, through various mechanisms. One of the mechanisms was disturbances in methylation metabolism which can be easily treated if it is proved. This study aims to evaluate the levels of S-adenosylmethionine [SAM] following sevoflurane anesthesia in neonates and to correlate the duration of sevoflurane exposure and S-adenosylmethionine levels.

Material and Methods: Sixty neonates were included in the study under general anesthesia. Pre- and postsevoflurane exposure arterial blood samples were collected in ethylenediamine tetraacetic acid vacutainers. Each sample was centrifuged at 1000 rpm for 10 min. Plasma was separated and stored at −80°C, then subjected to S-adenosylmethionine enzyme-linked immunoassay test for preand postsevoflurane exposure levels of SAM.

Results: The difference between the pre- and post-SAM values is not statistically significant and also with increasing the duration of sevoflurane exposure there was no reduction in the SAM levels ($r = 0.17$), and the correlation was not significant ($P = 0.18$).

Conclusion: Single exposure to sevoflurane does not impact SAM levels in neonates undergoing general anesthesia.

Keywords: Biomarker, neonates, sevoflurane

Introduction

Preclinical studies in rodents and primates have shown that anesthesia is neurotoxic to the developing brain after exposure in the neonatal period.[1] Most significant to modern neonatal clinical, practice is that all volatile anesthetics[1,2] and propofol[1,3] have been shown to induce the injury. Human studies on the mechanisms of the long-term adverse effects of general anesthesia are needed to improve the safety of general anesthesia. Still, they are hampered by ethical limitations specific to human research and a lack of specific biological markers that can be used in human studies to safely and objectively study such effects.[4]

Sevoflurane is a commonly used inhalational anesthetic, especially in pediatric surgery which might cause behavioral impairment in the developing brain[5-7] There is evidence indicating that the pathological mechanism may involve oxidative stress,[8] neuroapoptosis,[9] neuroinflammation,[10] altered synaptic properties,[11] and, especially, increased

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Tau[12] sevoflurane-induced neurotoxicity and found that neonatal exposure to sevoflurane disrupted glucose, lipid, and amino acids metabolism,[13] and many works indicated that abnormal methylation was closely associated with neurological diseases.[14] Inflammatory genes are regulated by methylation in neurodegenerative diseases.[15] Abnormal methylation occurred at the level of deoxyribonucleic acid [DNA] and the amino acid was concerned with neurological disability,[16] it has been recognized as a key regulator for many neurodegenerative diseases as well, including Alzheimer's disease[17] and methylation served as a crucial role between neuroinflammation and neurotoxicity.[18]

S-Adenosylmethionine [SAM][19] is the most important biomarker and occurs naturally in humans. It was discovered to be an active cofactor in biological methylation reactions by Cantoni [Annexure 2],[20] which acts as a methyl donor in many fundamental metabolic processes and is involved in the synthesis of various neurotransmitters in the brain. The decreasing SAM upregulates the homocysteine level, accelerating the neuropathology of neurodegenerative disorders by increasing beta-amyloid and Tau levels.[21,22] The levels of various metabolites of the methylation pathway are important for protection from the side effects of anesthesia, in 1942, it was recognized that the addition of methionine was used for the side effects of chloroform. Methionine affords protection from liver injury as a result of chloroform anesthesia. Neonatal exposure to sevoflurane may cause an alteration in the level of adenosylmethionine in animal studies.[23] To study the impact of sevoflurane on neonates' methylation, levels of biomarker SAM were measured using S-adenosylmethionine enzyme-linked immunoassay (SAM ELISA) Kit. This study was conducted to investigate the effect of sevoflurane anesthesia exposure on SAM and the correlation between exposure time and SAM levels.

Material and Methods

The study design was a prospective, single-group interventional study [Annexure 1]. After obtaining Institutional ethics clearance, the study was conducted on 60 neonates in our College Institute between January 2021 and August 2022. Term neonates undergoing surgery under general anesthesia, without prior exposure to general anesthesia or risk factors for neurologic injury surgery lasting more than 30 min, and hemodynamically stable neonates were recruited. Parental refusal, anticipating blood loss to exceed 10% of the subject blood volume, surgery lasting more than 180 min, difficult airway, small for age gestation [malnourished neonates], in severe sepsis, hemodynamically unstable, previous exposure to volatile general anesthesia or benzodiazepines as a neonate or in the third trimester in utero, any known neurological injury was excluded from the study. The primary objective was to evaluate the levels of SAM following sevoflurane anesthesia in neonates and the secondary objective was to correlate between duration of sevoflurane exposure and SAM. After obtaining informed consent from parents, neonates were included in the study; standard monitors were used. Preoperatively, adequate hydration was achieved with normal saline 10 mL/kg bolus and then maintenance with Ringer's lactate solution of 4 mL/kg/h. Thereafter preoxygenated with 100% O_2 for 3 min with 6 L of oxygen, intravenous induction was done with inj. thiopentone 5–7 mg/kg body weight, fentanyl 2 mcg/kg; once arterial blood 1–1.5 ml drawn using 2 ml 26 g needle from femoral artery under ultrasound guidance [linear small probe 5–7 MHz], sevoflurane [3%] was started. Atracuranium 0.5 mg/kg body weight was given once mask ventilation was confirmed. After 3 min of ventilation, once minimum alveolar concentration [MAC] was achieved intubated with uncuffed 3-mm-size endotracheal tube using 0 size Miller blade. Ventilation was delivered by pressure control ventilation mode by GE Carestation 750/750c (A1) ventilator at a respiratory rate and fresh gas flow (minimum of 1 lt/min). At the end of the surgery, after sevoflurane was discontinued when MAC of sevoflurane was 0, postsevoflurane arterial blood was drawn from the femoral artery before extubation. After labeling, pre- and postexposure blood samples were transferred to the pathology department. Baseline heart rate, mean arterial pressure, temperature, glucose levels, arterial blood gas analysis with preblood samples, and duration of sevoflurane were recorded. After surgery, patients were extubated and shifted to the neonatal intensive care unit. Sample collection – pre- and postsevoflurane exposure blood samples were collected in ethylenediamine tetraacetic acid vacutainers. Each sample was centrifuged at 1000 rpm for 10 min. Plasma was separated and stored at −80°C. Before the test, the samples were twofold diluted with phosphate buffer solution (PBS). Cell Biolabs' SAM ELISA kit is a competitive enzyme immunoassay developed for the detection and quantitation of SAM in plasma, serum, lysates, or other biological fluid samples. The kit has a detection sensitivity limit of 400 ng/mL S-adenosylmethionine-bovine serum albumin.

Preparation of reagents:

1. Preparation of SAM-coated plates:
   The number of wells to be used is calculated and the SAM conjugate provided in the kit is diluted with PBS in 1:100 concentration. Then, 100 µL of the diluted SAM conjugate is added to each 96-well protein binding plate well. The plate is incubated overnight at 4°C. The diluted SAM conjugate is removed using a blotting plate on paper towels. Wash wells 3 times with 200 µL of PBS

and blot them on paper towels to remove excess fluid. Add 200 µL of assay diluent to each well and block for 1 h at room temperature

2. Anti-SAM antibody and secondary antibody horseradish peroxides [HRP] conjugate: Immediately before use, dilute the anti-SAM antibody 1:500 and the secondary antibody HRP conjugate 1:1000 with assay diluent. Do not store diluted solutions.
3. 1× Wash buffer: Dilute the 10× wash buffer to 1× with deionized water. Stir to homogeneity.
4. Preparation of standards: SAM standards are prepared by serial dilution in the concentration range of 0–40 nM in assay diluent which is provided in the kit.

For tube-1, 10 µL of 1.2-µ SAM standard is diluted in 290 µL of assay diluent, totaling 300 µL. The SAM concentration of tube-1 will become 40 nM.

For tube-2, 150 µL of tube-1 SAM standard diluted with 150 µL of assay diluent. The SAM concentration of tube-2 will become 20 nM.

For tube-3, 150 µL of tube-2 SAM standard diluted with 150 µL of assay diluent. The SAM concentration of tube-3 will become 10 nM.

For tube-4, 150 µL of tube-3 SAM standard diluted with 150 µL of assay diluent. The SAM concentration of tube-4 will become 5 nM.

For tube-5, 150 µL of tube-4 SAM standard diluted with 150 µL of assay diluent. The SAM concentration of tube-5 will become 2.5 nM.

For tube-6, 150 µL of tube-5 SAM standard diluted with 150 µL of assay diluent. The SAM concentration of tube-6 will become 1.25 nM.

For tube-7, 150 µL of tube-6 SAM standard diluted with 150 µL of assay diluent. The SAM concentration of tube-7 will become 0.625 nM.

For tube-8, 150 µL of tube-7 SAM standard was discarded and only 150 µL of assay diluent were added to the tube. The SAM concentration of tube-8 will be 0.

Assay Protocol:

1. All the reagents are prepared as mentioned above.
2. Each test sample, SAM standard, and blank should be assayed in duplicate.
3. The assay diluent from the plate was removed and then 50 µL of the test sample or standard was added to the SAM conjugate coated plate, which incubated at room temperature for 10 min on an orbital shaker.
4. 50 µL of diluted anti-SAM Antibody was added to each tested well and incubated at room temperature for 1 h on an orbital shaker.
5. The microwell strips were washed 3 times with 250 µL 1× wash buffer per well with thorough aspiration between each wash.
6. 100 µL of the diluted secondary antibody HRP conjugate is added to each well and incubated at room temperature for 1 h on an orbital shaker.
7. The microwell strips were washed 3 times with 250 µL 1× wash buffer per well with thorough aspiration between each wash.
8. 100 µL of substrate solution was added to each well, including the blank wells, and incubated at room temperature on an orbital shaker.
9. 100 µL of stop solution was added into each well including the blank wells to stop the enzyme reaction.
10. The absorbance of each microwell is read on a spectrophotometer using 450 nm as the primary wavelength.

Sample size calculated depending on the outcome variable on the effect of sevoflurane on the level of S-adenosylmethionine, with a minimum difference of 1ng/g with standard deviation [SD] of 2.55 and 90% statistical power, 5% level of significance, the sample size of 60–70 was adequate for this single group pre–post design.[23]

For statistical analysis, data were entered in Microsoft Excel 2019 and were analyzed using MedCalc statistical software. The quantitative variables were expressed in mean, median, range, and standard deviation. The categorical variables were expressed in terms of percentages. The mean values of preanesthetic SAM values and pos-anesthesia SAM values were compared using a paired *t*-test. The *P* value of less than 0.05 was considered as significant. The correlation between the duration of sevoflurane exposure, arterial blood gas values, general random blood sugar [GRBS], heart rate, mean arterial pressure, and body temperature with postanesthesia SAM values was analyzed using correlation coefficient (*r*). The value of "*r*" greater than zero was considered a positive correlation and a value less than zero was considered a negative correlation. The "*P*" value of less than 0.05 was considered significant.

Results

The demographic profiles including age, weight, gender, American Society of Anaesthesiologists grade, and duration of

surgery were comparable. Eighty-two neonates were subjected to the study. There were 22 protocol violations in the group such as difficulty in taking arterial samples,[6] [maximum two attempts] inadequate sample collection,[6] clotting of samples,[2] hemolyzed,[6] and misplacing of samples[1] in the lab. Neonates were posted for different types of surgeries in that 24 were posted for tracheoesophageal fistula surgeries, 16 were with an anorectal malformation, 9 were for congenital diaphragmatic hernia repair, 8 were for intestinal obstruction laparotomy, and 4 were posted for meningomyelocele repair surgeries. Most neonates, about 55% were between 1 and 10 days old at the time of surgery [12.06 ± 9.71 days] [Table 1]. The average weight was 2.95 ± 0.63 kg [Table 2]. There were 30 male and 30 female neonates [Diagram 1].

There were not many changes in postsevoflurane values compared with presevoflurane values; the difference between pre- and postsevoflurane exposure of SAM values was not statistically significant. P =0.106 [Table 3 and Diagrams 2, 3] With the increase in the duration of sevoflurane exposure, there was no reduction in the SAM levels (r = 0.17), and the correlation was not significant (P = 0.18). [Diagram 4], whereas other parameters like GRBS, heart rate, hemoglobin, body temperature, pH, arterial oxygen tension, and arterial carbon dioxide tension values correlated with post-SAM samples, [Table 4 and Diagrams 5-8] and these parameters did not show any significance $P \geq 0.05$.

Table 1: Age distribution of cases

Age (in days)	No. of cases	Percentage
1–10	33	55.0
11–20	10	16.7
21–30	17	28.3
Total	60	100.0

Descriptive analysis – Mean age=12.06, Median=9 days, Standard deviation=9.71, Range=1–29 in days

Table 2: Weight distribution

Weight (in Kg)	No. of cases	Percentage
<2.5	15	25.0
2.5–3.0	19	31.7
3.01–3.5	15	25.0
3.51–4.0	10	16.7
>4.0	1	1.7
Total	60	100.0

Descriptive analysis-Mean – 2.95, Median – 2.95, Range – 1.8 – 4.2 in Kg. Standard deviation – 0.63 Kg

Discussion

In this study, neonates were exposed to sevoflurane of 1 MAC [1.5] for about 120–140 min compared to animal studies where animals were exposed to sevoflurane concentration of more than 1.5 for prolonged hours, which was more than 6 h and also; they were exposed repeatedly in a very shorter duration. The median duration of general anesthesia for children in the 1.5 million procedures in the National Anaesthesia Clinical Outcomes Registry (USA) was 57 min with infants having a median duration of 79 min.[24] Despite the protocol violations encountered during the study, such as difficulty in sample collection and handling, the main outcome measure of SAM levels remained consistent. Furthermore, the correlation between the duration of sevoflurane exposure and SAM levels was not significant (P = 0.18), suggesting that longer exposure to sevoflurane did not result in a decrease in SAM levels.

Human evidence overwhelmingly suggests that any effect of well-conducted pediatric anesthesia was insignificant or nonexistent. Reviewers of the GAS and PANDA[25] findings caution that some uncertainties remain while a brief anesthetic exposure appears safe. The GAS trial assessed the strongest human evidence on neurodevelopment and provided strong evidence that exposure to general anesthesia for just 1 h in infancy does not cause significant neurocognitive or behavioral deficits,[26] even in our study we did not find any impact of sevoflurane on SAM after exposure for about 120–140 min. We chose a biomarker study in neonates unlike previous cohort neurodegeneration follow-up cases as there was no

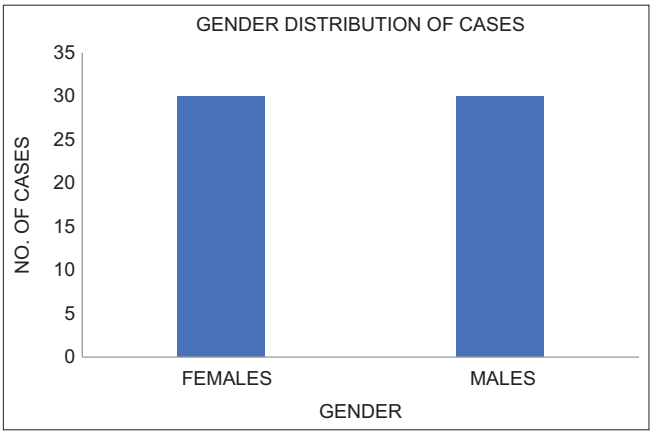

Diagram 1: Gender distribution

Table 3: Pretest VS post-test SAM values

Parameter	Mean	Standard deviation	Median	Range	Paired *t*- test	*P*
Pretest SAM LE levels VELS	21.32	5.20	20	6.67–33.33	t=-1.63	P=0.106 (not significant)
Post-test SAM levels	19.9	4.31	20	12–26.67		

Paired t-test, P value 0.106, SAM-S-adenosylmethionine. Difference=−1.42. Pooled standard deviation=4.77. Standard error=0.872. 95% CI=−3.147–0.307. Test statistics (t)=−1.629. DF=118. Significance level (P)=0.106

Table 4: Parameters

Parameter	Mean	Standard deviation	Median	Range	Correlation coefficient (R)	*P*
GRBS	107.63 MG	20.35 MG	102 MG	80–170 MG	− 0.19	0.13 (Not Significant)
Heart rate	133.8 BPM	10.5	132.5 BPM	112–164 BPM	− 0.09	0.49 (Not Significant)
MAP	50.3 MMHG	5.64	50 MMHG	38–68	0.23	0.06 (Not Significant)
Hemoglobin	14.74 GM	3.25	14.25 GM	9.5–23.4 GM	− 0.23	0.07 (Not Significant)
Body temperature	36.7	0.28	36.7	36.2–37.2	− 0.01	0.93 (Not Significant)
Duration of sevoflurane exposure	114.58 MIN	24.82	110 MIN	65–170 MIN	0.17	0.18 (Not Significant)
PH	7.35	0.06	7.36	7.21	-0.14	0.27 (Not Significant)
Po2	176.67	16.84	176	133-212	0.004	0.97 (Not Significant)
Pco2	34.38	5.36	34	21-46	0.28	0.028 (Not Significant)

P – not significant >0.05, correlation coefficient (r). GRBS – random blood sugar, MAP – mean arterial pressure, PO2 – partial pressure of oxygen, PCO2 –partial pressure of carbon dioxide, MG – milligram, BPM – beats per minute, mmHg – millimeters of mercury, MIN – minutes

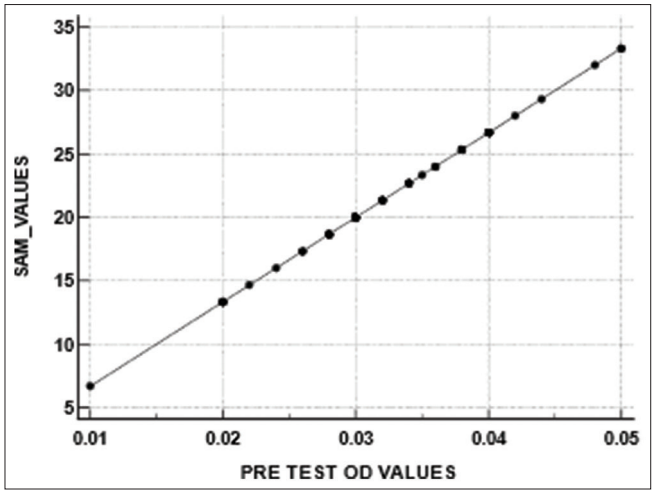

Diagram 2: Pretest OD values vs SAM levels

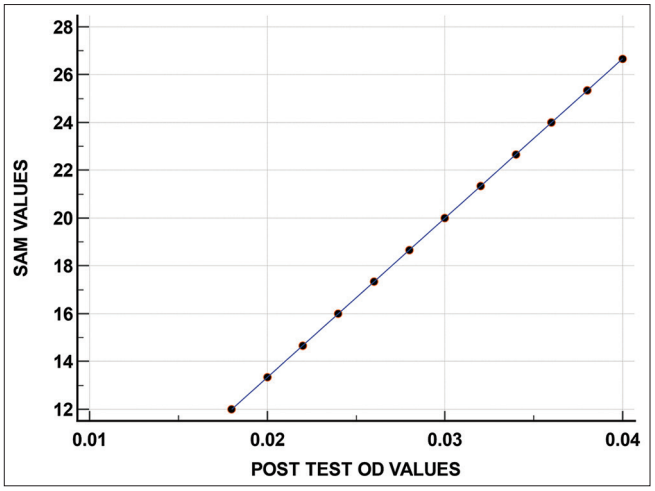

Diagram 3: Post-test OD values vs SAM levels

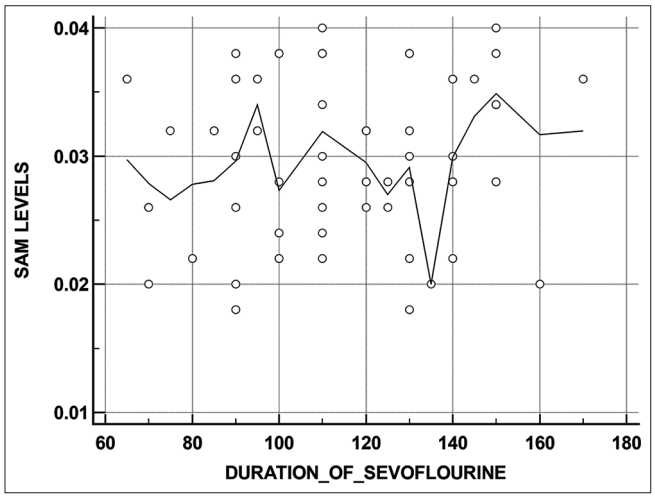

Diagram 4: Relation between duration of sevoflurane exposure and levels of s-adenosylmethionine

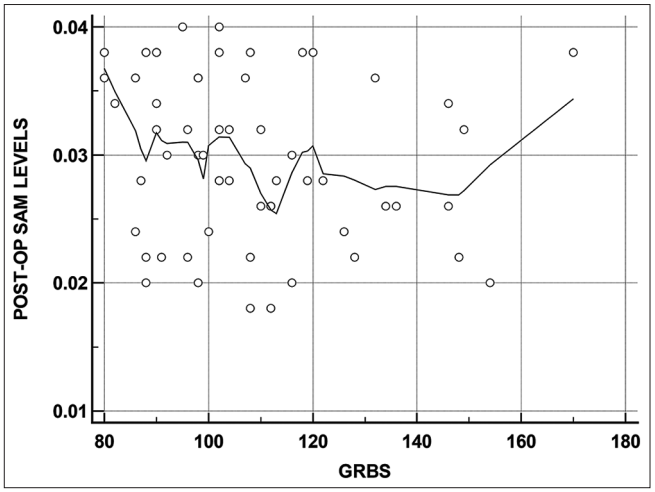

Diagram 5: Relation between GRBS and post levels of S-adenosylmethionine

follow-up process for years, and no difficulty in masking any intervention and to generalize the study group because of age, gender, weight, and any socioeconomic status. Parameters like GRBS, hemoglobin, body temperature, potential of hydrogen [PH], partial pressure of oxygen [PO2], and partial pressure of carbon dioxide [PCO2] are considered to minimize the bias in results. Given the complex nature of neurodevelopmental processes and multifactorial influences on brain function, the outcomes observed in this study were largely as predicted. However, it is important to acknowledge the potential limitations of extrapolating these findings to

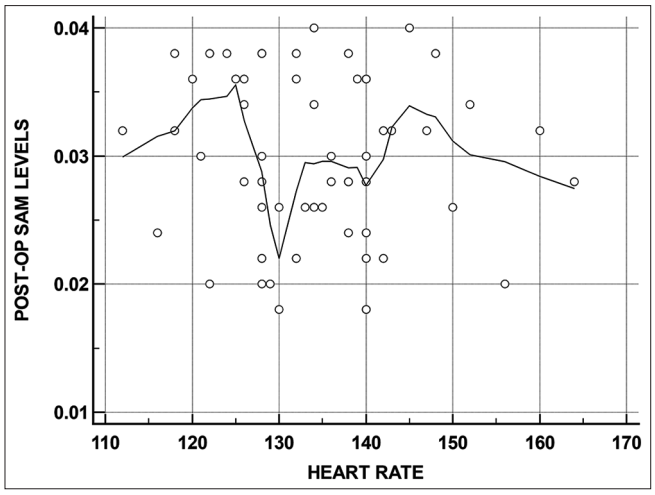

Diagram 6: Relation between heart rate and post levels of S-adenosylmethionine

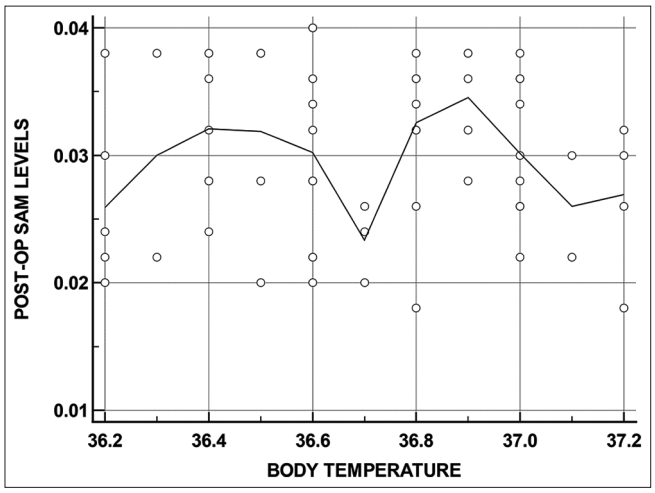

Diagram 7: Relation between body temperature and post levels of S-adenosylmethionine

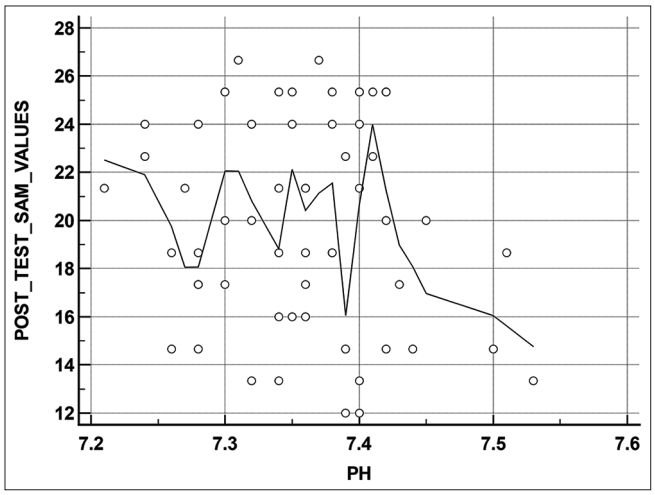

Diagram 8: Relation between PH and post levels of S-adenosylmethionine

broader conclusions about the long-term effects of anesthesia exposure in neonates.

Jialong Jiang *et al.*[23] investigated the mechanism of potential neurotoxicity on their offspring generation after sevoflurane anesthesia in adult animals during pregnancy based on metabolomics. SAM was inhibited dramatically after sevoflurane exposure as shown by enzyme-linked immunosorbent assay. In contrast, our study showed that the difference between the preexposure and postexposure SAM values was not statistically significant ($P = 0.106$), indicating that sevoflurane anesthesia did not have a significant impact on SAM levels in neonates.

Problems with the rodent experimental paradigm were that we cannot consider human babies as large rat pup.[27,28] It depends on many factors like duration of exposure to drugs[29,30] rapid brain growth,[31] lack of precise physiological monitoring, interspecies variation,[32] dose-response,[33,34] drug metabolism, and peak susceptibility. Also, lot of challenges in translating animal data to clinical settings like areas of exact periods of vulnerability, the dose required to cause injury [animals require high doses of intravenous anesthetics], and most studies have exposed animals to long periods of anesthesia.[35,36] Although our study did not find significant changes in SAM levels following one exposure, it contributes to understanding the biochemical response to anesthesia in neonates. While this does intersect with fields such as biochemistry and laboratory medicine, it also holds relevance to pediatric anesthesiology and developmental neurotoxicity research. Adding such biochemical markers in clinical studies can provide insights into the underlying mechanisms of anesthesia-induced neurotoxicity, thus contributing to improved patient care and safety. The findings from our study, while consistent with existing human evidence suggesting the safety of brief anesthesia exposure in infancy, do not definitively demonstrate the absence of potential adverse effects from prolonged or repeated exposures. Indeed, extrapolating our results to such scenarios, particularly in the absence of a control group and with a relatively short duration of exposure, would be premature and could lead to misconceptions. However, the discussion now includes a clarification regarding the limitations of extrapolating these findings to multiple exposures for a prolonged duration as this study was carried out for a relatively short (≤140 min) duration in healthy neonates. In contrast, studies in very sick neonates have to be considered.[37] As we develop a better understanding of these important questions in the preclinical and observational data, we will be able to more effectively design and implement the next wave of clinical trials. Collectively, it aims to avoid oversimplification and confusion by cautioning against drawing direct parallels with previous studies on different anesthetic agents.

Conclusion

Single exposure to sevoflurane anesthesia and duration of sevoflurane exposure in neonates undergoing general

anesthesia had no impact on postsevoflurane SAM levels compared to presevoflurane exposure SAM levels, also the in turn, no significant interference with methylation.

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Conflicts of interest

There are no conflicts of interest.

References

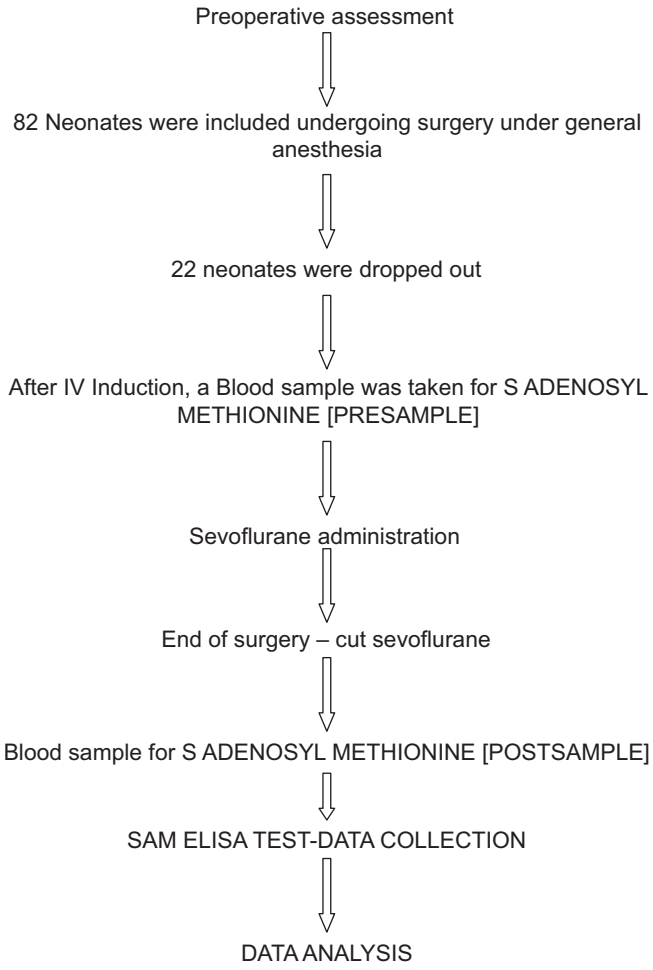
1. Sanders RD, Hassell J, Davidson AJ, Robertson NJ, Ma D. Impact of anesthetics and surgery on neurodevelopment: An update. Br J Anaesth 2013;110:53-72.
2. Liang G, Ward C, Peng J, Zhao Y, Huang B, Wei H. Isoflurane causes greater neurodegeneration than an equivalent exposure of sevoflurane in the developing brain of neonatal mice. Anesthesiology 2010;112:1325-34.
3. Cattano D, Young C, Straiko MM, Olney JW. Subanesthetic doses of propofol induce neuroapoptosis in the infant mouse brain. Anesth Analg 2008;106:1712-4.
4. Martynyuk E, Ju LS, Morey TE, Zhang JQ. Neuroendocrine, epigenetic, and intergenerational effects of general anesthetics. World J Psychiatr 2020;10:81-9.
5. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, *et al*. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70-4.
6. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, *et al*. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science 2000;287:1056-60.
7. Jevtovic-Todorovic V. Exposure of developing brain to general anesthesia: What is the animal evidence? Anesthesiology 2018;128:832-9.
8. Yufune S, Satoh Y, Akai R, Yoshinaga Y, Kobayashi Y, Endo S, *et al*. Suppression of ERK phosphorylation through oxidative stress is involved in the Mechanism underlying sevoflurane-induced toxicity in the developing brain. Sci Rep 2016;6:21859. doi: 10.1038/srep21859.
9. Kodama M, Satoh Y, Otsubo Y, Araki Y, Yonamine R, Masui K, *et al*. Neonatal desflurane exposure induces more robust neuroapoptosis than do isoflurane and Sevoflurane and impairs working memory. Anesthesiology 2011;115:979-91.
10. Wang Q, Zhao Y, Sun M, Liu S, Li B, Zhang L, *et al*. 2-Deoxy-d-glucoseattenuates sevoflurane-induced neuroinflammation through nuclear factor-kappa Bpathway *in vitro*. Toxicol *In Vitro* 2014;28:1183-9.
11. Zhang J, Dong Y, Zhou C, Zhang Y, Xie Z. Anesthetic sevoflurane reduces Levels of hippocalcin and postsynaptic density protein 95. Mol Neurobiol 2015;51:853-63.
12. Tao G, Zhang J, Zhang L, Dong Y, Yu B, Crosby G, *et al*. Sevoflurane induces tau phosphorylation and glycogen synthase kinase 3beta activation in young mice. Anesthesiology 2014;121:510-27.
13. Liu B, Gu Y, Xiao H, Lei X, Liang W, Zhang J. Altered metabolomic profiles may be associated with sevoflurane-induced neurotoxicity in neonatal rats. Neurochem Res 2015;40:788-99.
14. Miller A. The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med Rev 2008;13:216-26.
15. Nicolia V, Cavallaro RA, Lopez-Gonzalez I, Maccarrone M, Scarpa S, Ferrer I, *et al*. DNA methylation profiles of selected pro-inflammatory cytokines in Alzheimer disease. J Neuropathol Exp Neurol 2017;76:27-31.
16. Blanc RS, Richard S. Arginine methylation: The coming of age. Mol Cell 2017;65:824.
17. Do Carmo S, Hanzel CE, Jacobs ML, Machnes Z, Iulita MF, Yang J, *et al*. Rescue of early bace-1 and global DNA demethylation by S-adenosylmethionine reduces amyloid pathology and improves cognition in an Alzheimer's model. Sci Rep 2016;6:34051.
18. Zhu Y, Wang Y, Yao R, Hao T, Cao J, Huang H, *et al*. Enhanced neuroinflammation mediated by DNA methylation of the glucocorticoid receptor triggers cognitive dysfunction after sevoflurane anesthesia in adult rats subjected to maternal separation during the neonatal period. J Neuroinflammation 2017;14:1-16.
19. Sarris J, Papakostas GI, Vitolo O, Fava M, Mischoulon D. S-adenosylmethionine (SAMe) versus escitalopram and placebo in major depression RCT: Efficacy and effects of histamine and carnitine as moderators of response. J Affect Disord 2014;164:76-81.
20. Catoni GL. S-Adenosylmethionine; A new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. J Biol Chem 1953;204:403-16.
21. Li JG, Chu J, Barrero C, Merali S, Pratico D. Homocysteine exacerbates beta- amyloid pathology, tau pathology, and cognitive deficit in a mouse model of Alzheimer disease with plaques and tangles. Ann Neurol 2014;75:851-63.
22. Chung YC, Kruyer A, Yao Y, Feierman E, Richards A, Strickland S, *et al*. Hyperhomocysteinemia exacerbates Alzheimer's disease pathology by way of the beta-amyloid fibrinogen interaction. J Thrombo Haemost JTH 2016;14:1442-52.
23. Jiang J, Li S, Wang Y, Xiao X, Jin Y, Wang Y, *et al*. Potential neurotoxicity of prenatal exposure to sevoflurane on offspring: Metabolomics investigation on neurodevelopment and underlying mechanism. Int J Dev Neurosci 2017;62:46-53.
24. Bartels DD, McCann ME, Davidson AJ, Polaner DM, Whitlock EL, Bateman BT. Estimating paediatric general anaesthesia exposure: Quantifying duration and risk. Paediatr Anaesth 2018;28:520-7.
25. Chinn GA, Sasaki Russell JM, Sall JW. Is a short anesthetic exposure in children safe? time will tell: A focused commentary of the GAS and PANDA trials. Ann Transl Med 2016;4:408.
26. McCann ME, de Graaff JC, Dorris L, Disma N, Withington D, Bell G, *et al*. Neurodevelopmental outcome at 5 years of age after general anaesthesia or awake-regional anaesthesia in infancy (GAS): An international, multicentre, randomised, controlled equivalence trial. Lancet 2019;393:664-77.
27. Flurkey K, Currer JM, Harrison DE. Mouse models in aging research. In: Fox JG, Davisson MT, Quimby FW, Barthold SW, Newcomer CE, Smith AL, editors. The Mouse in Biomedical Research. 2nd ed. Burlington: Academic Press; 2007. p. 637-72.
28. Sengupta P. The laboratory rat: Relating its age with humans. Int J Prev Med 2013;4:624-30.
29. Shen X, Liu Y, Xu S, Zhao Q, Guo X, Shen R, *et al*. Early life exposure to sevoflurane impairs adulthood spatial memory in the rat. Neurotoxicology 2013;39:45-56.
30. Liu G, Zhu T, Zhang A, Li F, Qian W, Qian B. Heightened stress response and cognitive impairment after repeated neonatal sevoflurane exposures might be linked to excessive GABAAR-mediated depolarization. J Anesth 2016;30:834-41.
31. Dobbing J, Sands J. The brain growth spurt in various mammalian species. Early Hum Dev 1979;3:79-84.
32. Satomoto M, Satoh Y, Terui K, Miyao H, Takishima K, Ito M, *et al*. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in Fear conditioning in mice. Anesthesiology 2009;110:628-37.

33. Jia M, Liu WX, Yang JJ, Xu N, Xie ZM, Ju LS, *et al*. Role of histone acetylation in long-term neurobehavioral effects of neonatal exposure to sevoflurane in rats. Neurobiol Dis 2016;91:209-20.
34. Ji MH, Qiu LL, Yang JJ, Zhang H, Sun XR, Zhu SH, *et al*. Pre-administration of curcumin prevents neonatal sevoflurane exposure-induced neurobehavioral abnormalities in mice. Neurotoxicology 2015;46:155-64.
35. Disma N, O'Leary JD, Loepke AW, Brambrink AM, Becke K, Clausen NG, *et al*. Anesthesia and the developing brain: A way forward for laboratory and clinical research. Paediatr Anaesth 2018;28:758-63.
36. Montana MC, Evers AS. Anesthetic neurotoxicity: New findings and future directions. J Pediatr 2017;181:279-85.
37. Banerjee P, Rossi MG, Anghelescu DL, Liu W, Breazeale AM, Reddick WE, *et al*. Association between anesthesia exposure and neurocognitive and neuroimaging outcomes in long-term survivors of childhood acute lymphoblastic leukemia. JAMA Oncol 2019;5:1456-63.

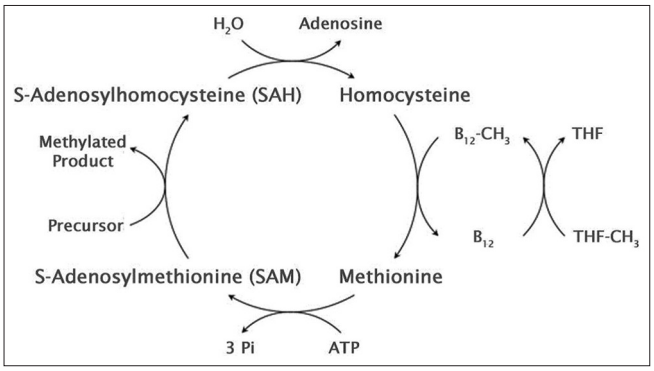
Annexure 1

Study design

Type of study: Single group interventional study



Annexure 2



Derived from the amino acid L-methionine through a metabolic pathway called the one-carbon cycle. S-Adenosylmethionine (SAM) is a methyl donor involved in the transfer of a methyl group to DNA, proteins, phospholipids, RNA, and neurotransmitters. Reactions that break down and regenerate SAM have been named the SAM cycle . SAM-dependent methylases use SAM as a substrate to yield s-adenosylhomocysteine (SAH), which is further broken down to homocysteine and adenosine by s-adenosylhomocysteine hydrolase. The homocysteine can be regenerated to methionine and finally SAM by methionine synthases.

S-adenosyl methionine occurs naturally in the human body. It may be synthesized from adenosine triphosphate and the alpha amino acid methionine. It was discovered to be an active cofactor in biological methylation reactions by Cantoni (Kresge 2005). As a physiological donor of methyl groups, it is involved in many cellular functions including the synthesis and metabolism of neurotransmitters.