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사람 배아 줄기세포로부터 얻어낸 신경 전구세포의 생존, 증식, 아포토시스, 분화에 대한 세보플루란 단기 노출의 영향

Effects of short-term exposure to sevoflurane on the survival, proliferation, apoptosis, and differentiation of neural precursor cells derived from human embryonic stem cells

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의학과 마취통증의학전공

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사람 배아 줄기세포로부터 얻어낸 신경 전구세포의 생존, 증식, 아포토시스, 분화에 대한 세보플루란 단기 노출의 영향

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Abstract

Effects of short-term exposure to sevoflurane on the survival, proliferation, apoptosis, and differentiation of neural precursor cells derived from human embryonic stem cells

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Purpose: Data from animal experiments suggest that exposure to general anesthetics in early life inhibits neurogenesis and causes long-term memory deficit. Regarding a short operating time and popularity of sevoflurane in pediatric anesthesia, it is important to verify effects of short period exposure to sevoflurane on developing brain.

Methods: We measured the effects of short-term exposure (2 h) to 3%, 6% or 8% sevoflurane, the most commonly used anesthetic, on neural precursor cells derived from human embryonic stem cells, SNUhES32. On days 1, 3, 5 and 7 post-treatment, cell survival, proliferation, apoptosis and differentiation were analyzed.

Results: Treatment with 6% sevoflurane increased cell viability (P = 0.046) and decreased apoptosis (P = 0.014) on day 5, but didn't last on day 7. Survival and apoptosis were not affected by 3% and 8% sevoflurane; there was no effect of

proliferation at any of the tested concentrations. The differentiation of cells exposed to 6% or 8% sevoflurane decreased on day 1 (P = 0.033 and 0.036 for 6% and 8% sevoflurane, respectively) but was again normalized on days 3–7.

Conclusion: The clinically relevant treatment with sevoflurane for 2 h induces no significant changes of the survival, proliferation, apoptosis and differentiation of human neural precursor cells, although supra-clinical doses of sevoflurane alter human neurogenesis transiently.

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keywords: anesthetics, general; human embryonic stem cells; neurogenesis; sevoflurane

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1. Introduction

Mounting preclinical data from experiments in rodents and non-human primates suggest that exposure to clinically common general anesthetics such as sevoflurane, isoflurane and propofol during early postnatal period inhibits neurogenesis and causes long-term memory deficit (1-6). Some clinical studies have also shown that general anesthesia in early childhood can lead to long-term behavioral abnormalities (7-10).

Neural precursor cells (NPCs) derived from human embryonic stem cells (hESCs) proliferate and differentiate to form mature neurons, astrocytes, or oligodendrocytes (11). The hESC-derived neurons established *in vitro* share morphologic, structural, physiological and functional traits with human neurons (12, 13). The efficient culture, expansion and differentiation of human stem cells *in vitro* (11, 14) have allowed research into the effect of anesthetics on the developing human brain, without the ethical problems posed by *in vivo* studies in human infants and children (15, 16).

Sevoflurane is one of the most commonly used anesthetics in general anesthesia. Due to its low blood–gas partition coefficient and nonpungency, sevoflurane provides smooth inhalation induction and is, in many cases, the anesthetic of choice in pediatric patients (17). In infants or children who undergo general anesthesia for surgery or diagnostic procedures, the duration of anesthesia is relatively shorter than in adults and generally under 2 h (18). Therefore, it is of prime importance to elucidate whether a short-term sevoflurane exposure may cause detrimental effects on the developing brain.

In this study, we aimed to investigate the effects of short-term exposure to

sevoflurane on human neurogenesis and therefore used cultured hESCs as a model system to evaluate the potential neurotoxicity of 2-h exposure to sevoflurane on NPCs derived from hESCs.

2. Materials and Methods

hESC line

Cells from the hESC line, SNUhES32 were maintained on 35-mm vitronectin-coated dishes under standard culture conditions (5% CO_2, 37°C) in an Essential 8 medium (Thermo fisher scientific, Carlsbad, CA, USA) with 2% Essential 8 supplement (Thermo fisher scientific). The medium was exchanged daily. Once a week, the cells were passaged by mechanical dispersion with flame-pulled Pasteur pipettes.

Derivation of NPCs from hESCs

NPCs were derived from hESCs according to the protocol our colleague had reported (14). The hESC colonies were washed with phosphate-buffered saline (PBS) and detached with collagenase for 40 min. Shortly thereafter, the detached colonies were transferred into a microtube containing ESC medium and allowed to settle to the bottom. The colonies were then transferred onto 60-mm bacterial dishes containing Essential 6 medium (Thermo fisher scientific). After culture with a medium change every other day for 5–7 days, the resulting EBs were transferred by pipetting to 35-mm dishes coated with Matrigel (BD Bioscience, San Jose, CA, USA). They were then cultured in neural precursor (NP) selection medium (Dulbecco's minimum essential medium/F12 medium supplemented with 0.5% N2 supplement, 1 mM L-glutamine, 1% non-essential amino acids, 50 U/ml penicillin, 50 μg/ml streptomycin and 0.1 mM 2-mercaptoethanol), which was changed every other day. After 5 days of selection, abnormal EBs recognized by their cystic form

were removed. The NP selection medium was replaced with NP expansion medium (add 20ng/ml bFGF, 0.5% N2 medium was replaced with 1% N2 medium). The NPs were cultured with a change of medium every other day. Neural structures having neural rosette or neural tube, seen as black clumps, appeared on day 5 and became larger by day 7. These were transferred to a 60-mm dish and incubated for ~2 days, during which time they formed spherical clumps. The spherical clumps were cut with flame-pulled Pasteur pipettes into smaller pieces to generate neural spheres. After 5 days of culture, the spheres had enlarged, forming spherical neural masses, which were purified in four time processes to yield a homogenous population.

Sevoflurane treatment

The NPCs were cut into small pieces mechanically and plated onto 96-well and 4-well plates for enzyme-linked immunosorbent assay (ELISA) and immunofluorescence staining, respectively. The cells were divided into two groups and incubated in modular incubator chambers (MIC 101, Billups-Rosenberg Inc., Del Mar, CA, USA). The NPCs of the sevoflurane group were exposed to 3%, 6% or 8% sevoflurane (Abbott Laboratories, Lake Bluff, IL, USA), provided by a vaporizer connected to the chamber (5% CO_2, 37°C), for 2 h. The cells in the chamber of the control group were cultured for 2 h without sevoflurane exposure. After treatment, the NPCs of both groups were cultured in neuronal differentiation medium, which was exchanged every other day. On days 1, 3, 5 and 7 post-exposure, the NPCs were analyzed for viability, proliferation, differentiation and apoptosis.

Determination of the medium concentration of sevoflurane by gas chromatography

Prior to the experiments, the concentrations of sevoflurane dissolved in the medium were determined using gas chromatography (GC) on an Agilent 7890A system (Agilent Technologies, Wilmington, DE, USA). A basal linear curve was obtained by GC measurement of four concentrations (0.25, 0.5, 1 and 5 mM) of sevoflurane in differentiation medium. After exposure of the cells to 3%, 6% and 8% sevoflurane (5% CO_2, 37°C) for 2 h, the media were analyzed by GC; the sevoflurane concentration was calculated based on the basal linear curve.

Cell viability analysis

Cell viability was determined colorimetrically using water-soluble tetrazolium salt (WST-1) assay kits (Roche Diagnostics, Basel, Switzerland). WST-1 was added to each well of the culture plates, which were then re-incubated for 2 h. WST-1 was reduced to dark yellow formazan by the action of cellular mitochondrial dehydrogenase. The reaction product was detected using a scanning multiwell spectrophotometer (ELISA reader) over the wavelength range of 420–480 nm. For each group, the assays were repeated for 12 samples.

Proliferation analysis

Cell proliferation was evaluated in a bromodeoxyuridine (BrdU)-based ELISA (Roche Diagnostics, Basel, Switzerland). BrdU is incorporated at thymidine positions during DNA synthesis and can thus be used to assess cell proliferation.

NP suspensions were incubated for 2 h with diluted BrdU solution. The cells were fixed and their DNA denatured using FixDenat solution. After a 30-min incubation, anti-BrdU-peroxidase antibody was added to the cells for 90 min; the cells were then washed with washing solution. The reaction product was quantified based on the absorbance in an ELISA reader. For each test or control group, 12 samples were analyzed for cell proliferation.

Apoptosis analysis

Cell apoptosis was quantified in a terminal deoxyribonucleotidyl transferase-dUTP nick end labeling (TUNEL) assay using a commercially available detection kit (Click-iT® TUNEL Alexa Fluor® imaging assay kit, Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. NPCs were fixed with 4% paraformaldehyde for 15 min and permeabilized with Triton X-100 for 20 min. The reaction cocktail, including terminal deoxyribonucleotidyl transferase and dUTP, was then added to the NPCs for 60 min. Cells undergoing apoptosis contained DNA strand breaks and thus incorporated dUTPs tagged with Alexa 594. Cell nuclei were then labelled with 4′, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA). Images of the TUNEL-stained cells were captured using an Olympus BX-60 fluorescence microscope (Olympus Corp., Tokyo, Japan). For each group, 12 random microscopic fields were imaged at 100× magnification.

Differentiation analysis

To evaluate neural cell differentiation, the differentiated cells were fixed with 4%

paraformaldehyde for 20 min, blocked for 1 h with 3% bovine serum albumin (BSA) and Triton X-100 in PBS and then incubated overnight at 4°C in 3% BSA containing anti-nestin and anti-β-III-tubulin antibodies. Nestin is an intermediate filament protein expressed in undifferentiated neural cells during development, whereas β-III tubulin is a microtubule protein present in differentiated neurons. The cells were incubated for 1 h with secondary antibodies tagged with Alexa 488 and 594 for nestin and β-III tubulin, respectively. After labelling of the cell nuclei with DAPI (Vector Laboratories), immuno-fluorecence labeled cells were examined by fluorescence microscopy. Twelve random microscopic fields at 100× magnification were imaged for each group.

Statistical analysis

Differences between the sevoflurane and control groups at each time point were investigated using an unpaired Student's t-test or the Mann–Whitney U-test for continuous variables with a normal or non-normal distribution, respectively. The data were also analyzed with a two-way repeated ANOVA to evaluate the difference between groups over time and the interactions group × time. All statistical analyses were performed using SPSS 19.0 software (SPSS Inc. Chicago, IL, USA). A P-value < 0.05 was considered to indicate statistical significance.

3. Results

Concentration of sevoflurane in the medium

A basal linear curve was established from the gas chromatographs of the four sevoflurane dilutions. After incubation of the cells for 2 h in 3%, 6% and 8% sevoflurane, the medium concentrations of the anesthetic were 0.369, 0.756 and 1.003 mM, respectively.

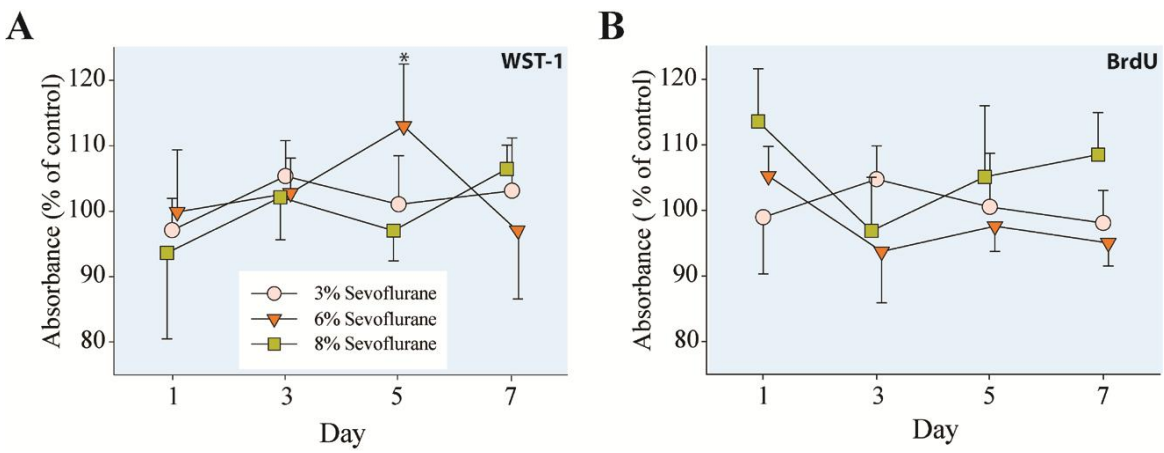

Figure 3.1 Survival and proliferation of neural precursor cells (NPCs) measured using an enzyme-linked immunosorbent assay. The results are expressed as a percentage of the control value. (A) A water-soluble tetrazolium salt (WST-1) assay showed that 6% sevoflurane increased NPC survival on day 5 but had no significant effect compared with the control at other time points. Exposure to 3% and 8% sevoflurane also had no effect on cell survival. (B) Sevoflurane treatment did not alter NPC proliferation at any time point, as demonstrated in a bromodeoxyuridine (BrdU) assay. Error bar = standard deviation of the mean, * $P < 0.05$.

Effects of sevoflurane on the survival of human NPCs

Generation of the dark yellow formazan reaction product in the WST-1 assay was an indicator of cell survival. The survival of NPCs exposed to 3% or 8% sevoflurane was not significantly different from the control at any time point during the week after treatment. Following the exposure of NPCs to 6%

sevoflurane, cell survival increased by 14% relative to control cells on day 5 (P = 0.046) (Figure 3.1A). However, on days 1 and 3 but also on day 7, survival of the 6% sevoflurane group did not differ significantly from that of the control group. There was no interaction group x time between control and any sevoflurane groups, indicating NPC exposure to the three sevoflurane concentrations did not significantly alter viability during the 7 days post-treatment.

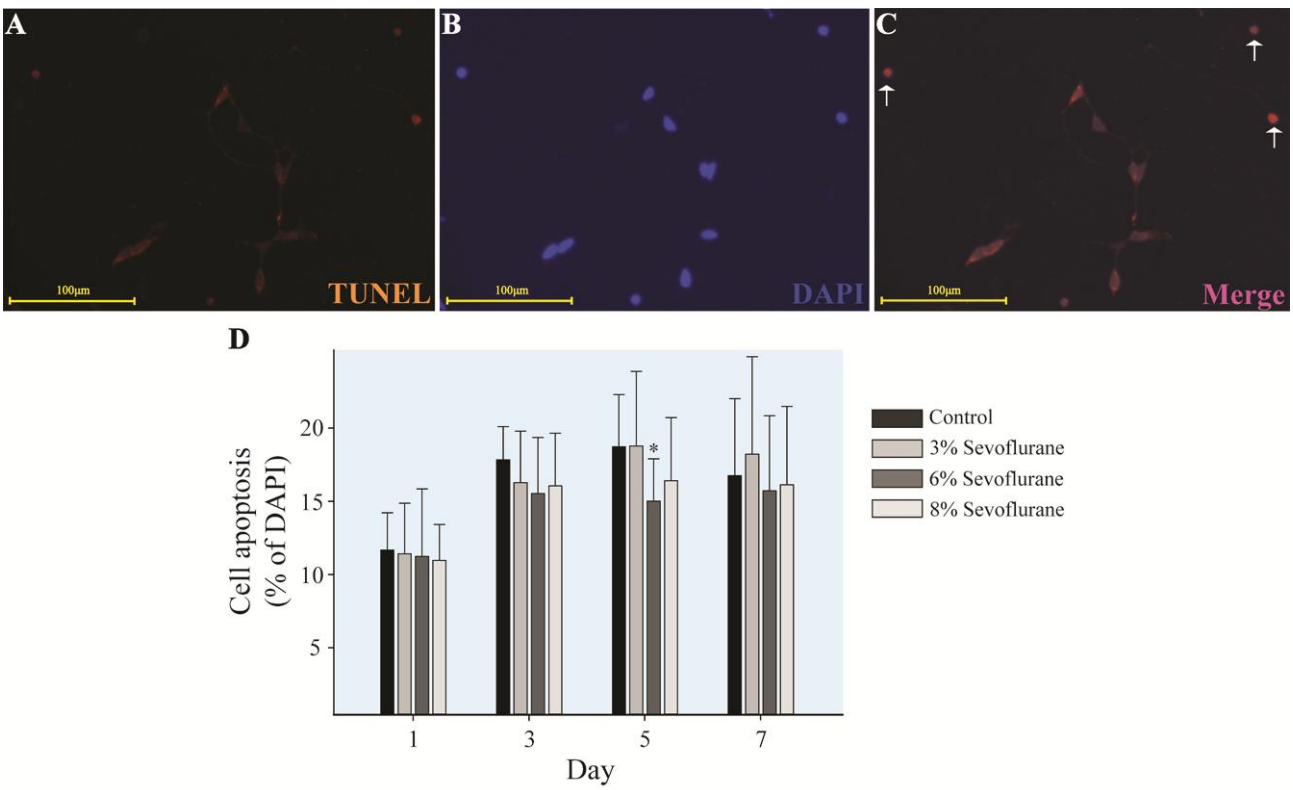

Figure 3.2 Terminal deoxyribonucleotidyl transferase-dUTP nickend labeling (TUNEL) assay for estimating NPC Apoptosis**.** (A) TUNEL-stained cells (B) 4′, 6-diamidino-2-phenylindole (DAPI)-stained nuclei. (A) and (B) show the same microscopic field. (C) TUNEL assay is based on the incorporation of dUTPs within nuclei undergoing apoptosis (white arrows).The proportion of apoptotic cells is expressed as a percentage of DAPI-positive nuclei. (D) Exposure to 6% sevoflurane decreased apoptosis on day 5 but had no effect on days 1, 3 and 7. Treatments with 3% or 8% sevoflurane did not cause any significant change in apoptosis on days 1–7. Error bar = standard deviation of the mean, * P < 0.05.

Effects of sevoflurane on the proliferation of human NPCs

The incorporation of BrdUs into DNA is proportional to the amount of cell proliferation. The sevoflurane groups did not show significant difference of proliferation compared with the control on any of the 4 days tested and over the 1-week post-exposure (Figure 3.1B).

Effects of sevoflurane on apoptosis by human NPCs

The proportion of TUNEL-stained (apoptotic) cells was determined as a percentage of the DAPI-positive nuclei (Figure 3.2A–C). NPC apoptosis in the 6% sevoflurane group was reduced by ~20% (P = 0.014) relative to the control group (Figure 3.2D) only on day 5, with no apparent effect on days 1, 3 and 7. In the 3% and 8% sevoflurane treatments, there was no significant change in apoptosis versus

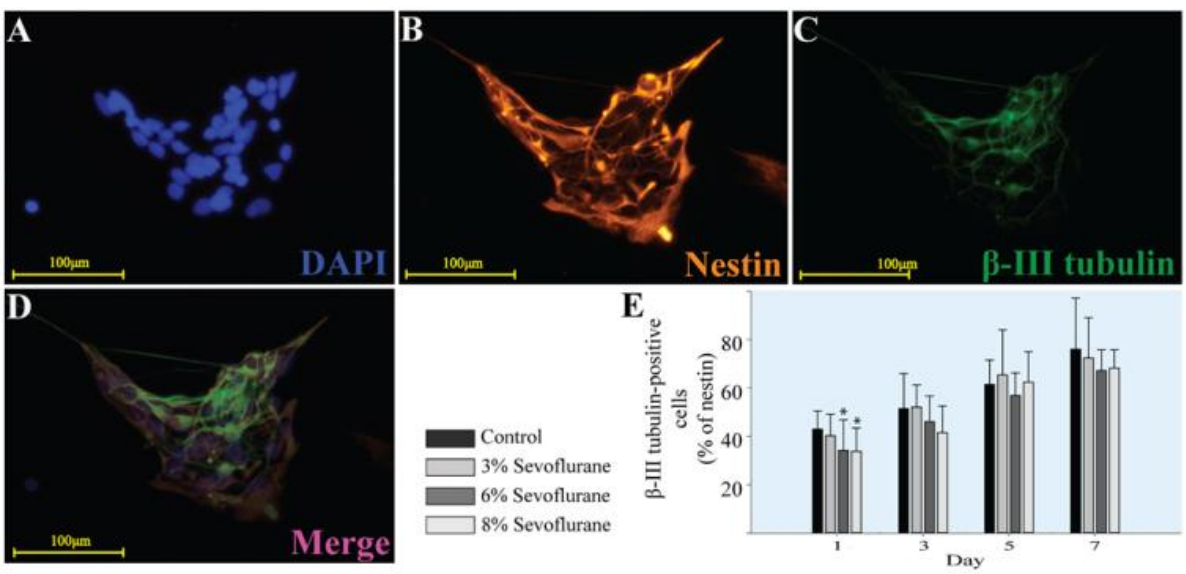

Figure 3.3 NPC differentiation examined by immunofluorescence staining. Same microscopic field: (A) DAPI-stained nuclei, (B) anti-nestin staining of undifferentiated neural cells, (C) anti-β-III tubulin staining of microtubule elements in differentiated neurons and (D) merge image. The ratio of β-III tubulin to nestin was used as an indicator of the relative rate of differentiation. (E) In cultures treated with 6% and 8% sevoflurane, the ratio significantly decreased on day 1 but not on days 3, 5 or 7. Treatment with 3% sevoflurane had no effect on the ratio on days 1–7. Error bar = standard deviation of the mean, * P < 0.05.

the control on days 1–7. Repeated ANOVA showed that exposure to any dose of sevoflurane did not significantly influence apoptosis during the 1-week experiment.

Effects of sevoflurane on human NPC differentiation

Differentiation from progenitor cell to neuron was determined from the ratio of the number of cells that stained with anti-β-III tubulin antibody versus the number of cells that stained positively with nestin (Figure 3.3A–D). In cells exposed for 2 h to 3% sevoflurane, there was no change in the ratio of β-III tubulin to nestin compared with the control on days 1, 3, 5 and 7 after treatment (Figure 3.3E). In the 6% and 8% sevoflurane groups, the ratios were significantly lower than those of the controls on day 1 ($P = 0.033$ and 0.036 for 6% and 8% sevoflurane, respectively) but not on days 3, 5 and 7. NPC differentiation significantly increased during the 1-week experiment in both the control and the experimental groups, with no effect induced by exposure to any of the three sevoflurane concentrations.

4. Discussion

This study investigated the effect of sevoflurane treatment on NPCs derived from hESCs. While animal studies using neural stem cells or neural precursor cells have demonstrated the neurotoxicity of general anesthetics, the effect of sevoflurane on neurogenesis of human stem cells has not been previously investigated (19).

The chosen sevoflurane concentrations, as well as exposure duration, reflected the clinical environment of general anesthesia during early-life surgery. Minimum alveolar concentrations (MACs) of sevoflurane in neonates and children younger than 12 years old are 3.3% and 2.5%, respectively (20). We therefore chose 3% sevoflurane as the dose to elucidate the effect of a clinical sevoflurane dose on NPCs. Additional treatments with 6% and 8% sevoflurane were designed to explore dose-dependent toxicity, which might reflect the risk of high anesthetic level over than 1 MAC. This situation might be encountered during the volatile induction of general anesthesia with sevoflurane or accidentally. According to a previous study analyzing pharmacokinetics of sevoflurane, arterial blood concentration of sevoflurane is equilibrated to about 1.9% during general anesthesia with 3.5% of sevoflurane in adult patients (21). Based on the physical properties of sevoflurane, 1.9% blood concentration means 9.861 mg of sevoflurane in 100 ml blood that is equal to 0.493 mM in molar concentration (21). Therefore, the concentrations of sevoflurane in the medium measured by gas chromatography (0.369 mM at 3.0% of sevoflurane) implicate that the sevoflurane treatment in our study could mimic, to some degree, a clinical situation under general anesthesia with sevoflurane.

Our results showed that a 2-h exposure of NPCs to 3% and 8% sevoflurane did

not affect the survival, proliferation and apoptosis of human NPCs 1, 3, 5 and 7 days after treatment; this differed from the results obtained in animal studies, in which the neurotoxicity of general anesthetics was consistently demonstrated. In those investigations, the concentration and duration of general anesthesia were the two main factors determining neurotoxicity (22-24). Therefore, a longer than 2 h or repeated exposure to sevoflurane may be a favorable condition to show anesthetic toxicity (25, 26). However, the effects of short-term sevoflurane treatment deserve to be investigated, considering the short duration of early life anesthesia. In addition, the level of anesthetic treatment in our study was the concentration that could induce considerable neural death in previous animal studies (4, 27).

In our study, on day 5 after exposure to 6% sevoflurane, a decrease in apoptosis and an increase in cell viability were determined. These effects were not observed on day 3 and were not maintained until day 7, suggesting that they were cumulative, slow to emerge, but also transient. A neuroprotective effect of general anesthetics has been reported in several studies (23, 28-31). However, in animal studies, the enhancement of cell survival by general anesthetics was achieved with a lower concentration or with a shorter exposure duration than tested in our study (30). It was reported that a 1-h exposure to 1 MAC or 1.5 MAC sevoflurane increased the number of viable neural stem cells isolated from rat embryos (23).

Our results suggest that the resistance of human neurogenesis to sevoflurane anesthesia is more considerable than expected from previous animal research. These findings may reflect the ontogenetic differences in the neural development of humans versus that of animals. The developing brain is susceptible to anesthetic neurotoxicity during growth spurt period (24, 32, 33). However, for example in

rodents, maturation of the nervous system is largely postnatal, whereas in humans it is prenatal (34). Drug potency and vulnerability also differ between animals and humans. In fact, the common aspects of the neurotoxicity pathway of sevoflurane among species remain largely unknown.

We also evaluated the differentiation of human NPCs exposed to sevoflurane. Seven days after a 2-h treatment with 3% sevoflurane, there was no difference in the degree of differentiation compared with the control. This finding agrees with a previous study in which clinically relevant concentrations of sevoflurane did not alter the differentiation of rat hippocampal neural stem cells (23). In the present study, supraclinical doses (6% and 8%) of sevoflurane decreased the rate of differentiation on day 1, but the effect disappeared by day 3 until day 7. Moreover, This transient effect of sevoflurane on the differentiation of NPCs is quite similar to the isoflurane effect on the NPCs differentiation in our previous study.(35) On the other hand, an animal study reported 4% sevoflurane hindered the differentiation of mouse embryonic stem cells for over a week (36). In comparison to animal cells, human tissues seem to regain their capacity for normal differentiation in response to treatment with high sevoflurane dose.

The few human clinical studies reporting anesthetic neurotoxicity were retrospective and were vulnerable to limitations such as inadequate sample size, bias and the lack of standardized outcome measure (8-10). The General Anesthesia compared to Spinal anesthesia (GAS) study is one of the several ongoing large-scale prospective studies that seek to overcome the limits of previous clinical studies (7). Its advantages are the investigation of a single general anesthetic agent in the setting of one surgical procedure, such that biases and logistical problems

can be avoided. The preliminary report of the GAS study concluded that general and regional anesthesia are functionally equivalent, which is consistent with our results (37). The consensus between preclinical and clinical human studies is meaningful for parents and clinicians. Although the GAS study is still in progress and the primary outcome has yet to be assessed, its preliminary conclusions indicate that general anesthesia with sevoflurane under clinical conditions need not be avoided (38, 39).

The current work has several limitations. First, the cells were derived from a cell line (hESC) that may differ from precursor cells generated and regulated within a living organism. Thus, neurogenesis, a complex, dynamic process that occurs over a lifetime, could not be reproduced perfectly. It is an innate weak point for studies using cultured stem cells. The signals for neural progenitor or stem cell proliferation, apoptosis and differentiation are closely correlated and developmentally regulated. Therefore, sevoflurane sensitivity may vary depending on the developmental stage (40, 41) whereas we investigated the changes in hESC-derived NPCs only for a week. Further studies are needed to determine the long-term effects of sevoflurane on NPCs or to analyze the neurotoxicity of sevoflurane during the different stages of human neurogenesis. Second, we did not evaluate the effects of long-term (> 2 h) exposure to sevoflurane in which significant neurotoxicity could occur. Previous animal studies have identified possible mechanisms of sevoflurane neurotoxicity. The *let-7a*-Lin 28 signaling pathway and ERK phosphorylation were demonstrated to be involved in sevoflurane-induced toxicity in the developing brain (36, 42). Although we did not investigate neurotoxic pathway of sevoflurane because there was no significant toxic effect

persisting 7 days after treatment, mechanistic studies are needed to identify and prevent a potential neurotoxicity after sevoflurane exposure for a longer duration than 2 hours.

An advantage of our study was that the effects of sevoflurane were examined at four different time points rather than a single one, which increased the reliability of the assays and allowed comprehensive inferences to be drawn from the accumulated data (23, 36, 43). Despite a few differences at specific time points, 2 h exposure to sevoflurane was shown not to affect the overall survival, proliferation, apoptosis and differentiation of human NPCs during the 1-week post-treatment. Moreover, as the first *in vitro* study to investigate the effects of sevoflurane on human tissues, our work provides powerful and essential information. In human cells unlike in animal cells, not only cell viability but also cell differentiation was relatively unaffected by sevoflurane. A previous clinical study demonstrated that multiple exposures, but not a single exposure, to general anesthetics caused long-term learning disabilities (10). These findings must be taken into account in further studies on the effects of anesthetics on human neurogenesis, by the inclusion not only of dose-response analyses but also of different clinical conditions, such as multiple exposures or combinations of anesthetic agents.

In conclusion, we report that clinically relevant doses of sevoflurane do not affect the survival, proliferation, apoptosis, or differentiation of human NPCs. Although supraclinical doses exerted toxic or protective effects on human neural cells, these were limited and transient.

5. References

1. Loepke AW, Istaphanous GK, McAuliffe JJ, 3rd, Miles L, Hughes EA, McCann JC, et al. The effects of neonatal isoflurane exposure in mice on brain cell viability, adult behavior, learning, and memory. Anesth Analg. 2009;108(1):90-104.
2. Fang F, Xue Z, Cang J. Sevoflurane exposure in 7-day-old rats affects neurogenesis, neurodegeneration and neurocognitive function. Neuroscience bulletin. 2012;28(5):499-508.
3. Fujimoto S, Ishikawa M, Nagano M, Sakamoto A. Influence of neonatal sevoflurane exposure on nerve development-related microRNAs and behavior of rats. Biomedical research. 2015;36(6):347-55.
4. Zheng H, Dong Y, Xu Z, Crosby G, Culley DJ, Zhang Y, et al. Sevoflurane anesthesia in pregnant mice induces neurotoxicity in fetal and offspring mice. Anesthesiology. 2013;118(3):516-26.
5. 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(5):979-91.
6. Wang SQ, Fang F, Xue ZG, Cang J, Zhang XG. Neonatal sevoflurane anesthesia induces long-term memory impairment and decreases hippocampal PSD-95 expression without neuronal loss. European review for medical and pharmacological sciences. 2013;17(7):941-50.
7. Sun L. Early childhood general anaesthesia exposure and neurocognitive development. British journal of anaesthesia. 2010;105 Suppl 1:i61-8.
8. DiMaggio C, Sun LS, Kakavouli A, Byrne MW, Li G. A retrospective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children. Journal of neurosurgical anesthesiology. 2009;21(4):286-91.
9. Kalkman CJ, Peelen L, Moons KG, Veenhuizen M, Bruens M, Sinnema G, et al. Behavior and development in children and age at the time of first anesthetic exposure. Anesthesiology. 2009;110(4):805-12.
10. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology. 2009;110(4):796-804.
11. Brokhman I, Gamarnik-Ziegler L, Pomp O, Aharonowiz M, Reubinoff BE, Goldstein RS. Peripheral sensory neurons differentiate from neural precursors derived from human embryonic stem cells. Differentiation; research in biological diversity. 2008;76(2):145-55.
12. Johnson MA, Weick JP, Pearce RA, Zhang SC. Functional neural development from human embryonic stem cells: accelerated synaptic activity via astrocyte coculture. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007;27(12):3069-77.
13. Cho MS, Lee Y-E, Kim JY, Chung S, Cho YH, Kim D-S, et al. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(9):3392-7.
14. Cho MS, Hwang DY, Kim DW. Efficient derivation of functional dopaminergic neurons from human embryonic stem cells on a large scale. Nat Protoc. 2008;3(12):1888-94.
15. Zhou Z, Ma D. Anaesthetics-induced neurotoxicity in developing brain: an update on preclinical evidence. Brain sciences. 2014;4(1):136-49.
16. Wang C, Liu F, Patterson TA, Paule MG, Slikker W, Jr. Utilization of neural stem cell-derived models to study anesthesia-related toxicity and preventative approaches.

Molecular neurobiology. 2013;48(2):302-7.
17. Lerman J. Sevoflurane in pediatric anesthesia. Anesth Analg. 1995;81(6 Suppl):S4-10.
18. Fingar KR, Stocks C, Weiss AJ, Steiner CA. Most Frequent Operating Room Procedures Performed in U.S. Hospitals, 2003-2012: Statistical Brief #186. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Rockville (MD)2006.
19. Skalova S, Svadlakova T, Shaikh Qureshi WM, Dev K, Mokry J. Induced pluripotent stem cells and their use in cardiac and neural regenerative medicine. Int J Mol Sci. 2015;16(2):4043-67.
20. Hatch DJ. New inhalation agents in paediatric anaesthesia. British journal of anaesthesia. 1999;83(1):42-9.
21. Lu CC, Tsai CS, Ho ST, Chen WY, Wong CS, Wang JJ, et al. Pharmacokinetics of sevoflurane uptake into the brain and body. Anaesthesia. 2003;58(10):951-6.
22. Zhang Y, Dong Y, Zheng H, Shie V, Wang H, Busscher JJ, et al. Sevoflurane inhibits neurogenesis and the Wnt-catenin signaling pathway in mouse neural progenitor cells. Current molecular medicine. 2013;13(9):1446-54.
23. Nie H, Peng Z, Lao N, Dong H, Xiong L. Effects of Sevoflurane on Self-Renewal Capacity and Differentiation of Cultured Neural Stem Cells. Neurochemical Research. 2013;38(8):1758-67.
24. Sinner B, Becke K, Engelhard K. General anaesthetics and the developing brain: an overview. Anaesthesia. 2014;69(9):1009-22.
25. Zhang X, Liu S, Newport GD, Paule MG, Callicott R, Thompson J, et al. In Vivo Monitoring of Sevoflurane-induced Adverse Effects in Neonatal Nonhuman Primates Using Small-animal Positron Emission Tomography. Anesthesiology. 2016;125(1):133-46.
26. Xiao H, Liu B, Chen Y, Zhang J. Learning, memory and synaptic plasticity in hippocampus in rats exposed to sevoflurane. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience. 2016;48:38-49.
27. Wang S, Peretich K, Zhao Y, Liang G, Meng Q, Wei H. Anesthesia-induced neurodegeneration in fetal rat brains. Pediatric research. 2009;66(4):435-40.
28. Sun X, Fang B, Zhao X, Zhang G, Ma H. Preconditioning of Mesenchymal Stem Cells by Sevoflurane to Improve Their Therapeutic Potential. PLoS ONE. 2014;9(3):e90667.
29. Lucchinetti E, Zeisberger SM, Baruscotti I, Wacker J, Feng J, Zaugg K, et al. Stem cell-like human endothelial progenitors show enhanced colony-forming capacity after brief sevoflurane exposure: preconditioning of angiogenic cells by volatile anesthetics. Anesth Analg. 2009;109(4):1117-26.
30. Wei H, Liang G, Yang H. Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity. Neuroscience letters. 2007;425(1):59-62.
31. Zhao P, Zuo Z. Isoflurane preconditioning induces neuroprotection that is inducible nitric oxide synthase-dependent in neonatal rats. Anesthesiology. 2004;101(3):695-703.
32. Jevtovic-Todorovic V, Boscolo A, Sanchez V, Lunardi N. Anesthesia-induced developmental neurodegeneration: the role of neuronal organelles. Frontiers in neurology. 2012;3:141.
33. Loepke AW, Soriano SG. An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function. Anesth Analg. 2008;106(6):1681-707.
34. Rice D, Barone S, Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environmental health perspectives. 2000;108 Suppl 3:511-33.
35. Sohn HM, Kim HY, Park S, Han SH, Kim JH. Isoflurane decreases proliferation and differentiation, but none of the effects persist in human embryonic stem cell-derived

neural progenitor cells. Journal of anesthesia. 2017;31(1):36-43.
36. Yi X, Cai Y, Zhang N, Wang Q, Li W. Sevoflurane inhibits embryonic stem cell self-renewal and subsequent neural differentiation by modulating the let-7a-Lin28 signaling pathway. Cell and tissue research. 2016;365(2):319-30.
37. Davidson AJ, Disma N, de Graaff JC, Withington DE, Dorris L, Bell G, et al. Neurodevelopmental outcome at 2 years of age after general anaesthesia and awake-regional anaesthesia in infancy (GAS): an international multicentre, randomised controlled trial. Lancet. 2016;387(10015):239-50.
38. Pound P, Bracken MB. Is animal research sufficiently evidence based to be a cornerstone of biomedical research? Bmj. 2014;348:g3387.
39. Hansen TG. Anesthesia-related neurotoxicity and the developing animal brain is not a significant problem in children. Paediatric anaesthesia. 2015;25(1):65-72.
40. LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron. 1995;15(6):1287-98.
41. Ryu JR, Hong CJ, Kim JY, Kim EK, Sun W, Yu SW. Control of adult neurogenesis by programmed cell death in the mammalian brain. Molecular brain. 2016;9:43.
42. 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. Scientific reports. 2016;6:21859.
43. Zhou L, Wang Z, Zhou H, Liu T, Lu F, Wang S, et al. Neonatal exposure to sevoflurane may not cause learning and memory deficits and behavioral abnormality in the childhood of Cynomolgus monkeys. Scientific reports. 2015;5:11145.

요약(국문초록)

사람 배아 줄기세포로부터 얻어낸 신경 전구세포의 생존, 증식, 아포토시스, 분화에 대한 세보플루란 단기 노출의 영향

목적: 동물 세포를 이용한 기존 실험들의 결과에 따르면 어린 시기에 노출된 전신마취제에 의해 신경 발달 과정을 저해되고 장기적인 기억력 감퇴 등이 유발될 수 있다. 세보플루란은 소아 마취에서 가장 흔하게 사용되는 전신마취제 중 하나이며, 소아 수술의 경우 성인 수술에 비해 비교적 수술 및 마취 시간이 짧기 때문에 세보플루란의 단기 노출이 발달과정 중의 뇌에 미치는 영향을 파악하는 것은 임상적으로 매우 중요하다.

방법: 사람 배아 줄기세포(SNUhES32)로부터 분화시킨 신경 전구세포를 3%, 6% 그리고 8%의 세보플루란에 각각 노출 시킨 뒤 그 영향을 파악하고자 하였다. 세보플루란 노출 후 1, 3, 5 그리고 7일 째 되는 시점에 세포의 생존, 증식, 아포톱시스, 그리고 분화 정도를 측정 및 분석하였다.

결과: 6% 세보플루란 처치 후 5일 째 세포의 생존이 증가하고 (P =

0.046) 아포톱시스가 감소하였다 (P = 0.014). 하지만 이러한 효과는 노출 후 7일 째 까지 지속되지는 않았다. 반면 3% 및 8%의 세보플루란 노출은 세포의 생존 및 아포톱시스에 영향을 미치지 않았다. 세포 증식의 경우, 어떠한 농도의 세보플루란 노출에 의해서도 유의한 증식의 변화는 유발되지 않았다. 노출 후 1일 째, 세보플루란 6% 와 8% 그룹에서 유의한 세포 분화의 저하가 발생하였다 (6% 와 8% 세보플루란 그룹에서 각각 P = 0.033 그리고 0.036). 세포 분화는 노출 후 3일 이후로는 정상화 되었다.

결론: 임상적인 수준의 단기 세보플루란 노출은 사람 신경 전구세포의 생존, 증식, 아포톱시스 그리고 분화에 유의한 영향을 미치지 않으며, 임상적 수준 이상의 높은 농도의 세보플루란 노출은 사람의 신경 발달 과정에 일시적인 변화를 유발할 수 있다.

주요어: 전신마취제, 사람 배아 줄기세포, 신경 발달 과정, 세보플루란

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