

Effects of Anesthetic Isoflurane and Desflurane on Human Cerebrospinal Fluid A β and τ Level

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ABSTRACT

Background: Accumulation of β -amyloid protein (A β) and tau protein is the main feature of Alzheimer disease neuro-pathogenesis. Anesthetic isoflurane, but not desflurane, may increase A β levels *in vitro* and in animals. Therefore, we set out to determine the effects of isoflurane and desflurane on cerebrospinal fluid (CSF) levels of A β and tau in humans.

Methods: The participants were assigned into spinal anesthesia (N = 35), spinal plus desflurane anesthesia (N = 33), or spinal plus isoflurane anesthesia (N = 38) group by

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What We Already Know about This Topic

- Isoflurane, but not desflurane, increases β -amyloid proteins (A β) in animals, and this may participate in postoperative cognitive effects of anesthesia in humans

What This Article Tells Us That Is New

- In 106 patients randomized to receive spinal anesthesia for surgery without general anesthetics or with isoflurane or desflurane, isoflurane was associated with an increase in cerebrospinal fluid concentrations of A β 40 and desflurane with a decrease in A β 42
- This clinical work parallels observations in animals, but its relevance to cognition after surgery remains uncertain

randomization using computer-generated lists. Pre- and postoperative human CSF samples were obtained through an inserted spinal catheter. The levels of A β (A β 40 and A β 42) and total tau in the CSF were determined.

Results: Here, we show that isoflurane, but not desflurane, was associated with an increase in human CSF A β 40 levels (from 10.90 to 12.41 ng/ml) 24 h after the surgery under anesthesia compared to spinal anesthesia (from 11.59 to 11.08 ng/ml), $P = 0.022$. Desflurane, but not isoflurane, was associated with a decrease in A β 42 levels 2 h after the surgery under anesthesia (from 0.39 to 0.35 ng/ml) compared to spinal anesthesia (from 0.43 to 0.44 ng/ml), $P = 0.006$. Isoflurane and desflurane did not significantly affect the tau levels in human CSF.

Conclusions: These studies have established a system to study the effects of anesthetics on human biomarkers associated with Alzheimer disease and cognitive dysfunction. These findings have suggested that isoflurane and desflurane may have different effects on human CSF A β levels.

AN estimated 234 million patients worldwide undergo anesthesia and surgery each year. Even though the incidence of Alzheimer disease (AD) may not be increased by anesthesia,¹ the age of AD onset was reported to be inversely related to cumulative exposure to anesthesia and surgery before the age of 50 yrs.² Several other studies have also suggested general anesthesia as one of the risk factors for AD.^{3,4} Opposing findings, however, do exist,⁵⁻⁷ and therefore more research, especially human studies aimed to define the role of anesthesia in AD neuropathogenesis, is necessary.^{8,9}

Excessive production and/or accumulation of β -amyloid protein ($A\beta$), predominantly including $A\beta_{40}$ and $A\beta_{42}$, plays a fundamental role in the neuropathogenesis of AD.^{10–13} In addition, recent studies have suggested the contribution of $A\beta_{40}$ in AD neuropathogenesis.^{14,15} Furthermore, $A\beta_{40}$ has been shown to induce caspase activation and apoptosis^{16–18} and to impair synaptic plasticity and memory function.^{19,20}

In addition to $A\beta$, the accumulation, abnormal aggregation, and deposition of tau play a key role in AD neuropathogenesis.²¹ As the major protein component of intraneuronal neurofibrillary tangles, elevation of total tau levels (two- to threefold) is seen in the cerebrospinal fluid (CSF) of AD patients compared to nondemented elderly adults.^{22,23} Moreover, a reduced ratio of $A\beta_{42}$ to tau in the CSF has been suggested as one of the biomarkers of AD.^{23,24}

Several studies have suggested that the commonly used inhalation anesthetic isoflurane can increase $A\beta$ accumulation in cultured cells, neurons, and animals.^{9,25–29} The isoflurane has been shown to increase levels of phosphorylated, but not total, tau in brain tissues of mice.³⁰ In contrast, another commonly used inhalation anesthetic desflurane has been shown not to increase $A\beta$ levels in cultured cells.³¹ However, the *in vivo* relevance of these acute effects of isoflurane on $A\beta$ levels in humans has not yet been assessed. In this study, we set out to determine the effects of surgery under spinal (S) anesthesia alone, spinal plus desflurane (SD) anesthesia, and spinal plus isoflurane (SI) anesthesia on the acute changes in CSF $A\beta$ levels in human participants. We also assessed the effects of surgery under S, SD, and SI anesthesia on total tau levels in human CSF and calculated the ratios of $A\beta_{40}$ or $A\beta_{42}$ to tau. We hypothesized, based on the *in vitro* and animal studies, that isoflurane, but not desflurane, would increase the human CSF $A\beta$ levels.

Materials and Methods

The Participants

The protocol was approved by the research Ethics Committee in the Capital Medical University in Beijing, P.R. China, and the institutional review board of Partners Human Research Committee, Boston, Massachusetts. Eligible participants were American Society of Anesthesiologists I or II patients between 60 and 75 yrs of age who had either lower extremity or lower abdominal surgery under S anesthesia alone, SD anesthesia, or SI anesthesia in the Beijing Friendship Hospital, Capital Medical University, Beijing, China. The exclusion criteria included (1) patients below 64 yr and over 73 yr of age; (2) patients who cannot accept continuous spinal anesthesia; (3) patients with neurological or psychiatric disorders; (4) American Society of Anesthesiologists III or IV patients; (5) patients with contraindications for spinal anesthesia and the local anesthetics; (6) patients with serious anxiety and depression or with pulmonary infection; pulmonary embolism; cerebral

thrombosis/ hemorrhage; (7) patients participating in other clinical trials.

The Anesthesia

The study has a parallel arm trial design. After obtaining the signed consent form, all the participants had spinal anesthesia with a spinal anesthesia catheter (Fajunk, Geisingen, Germany) and were assigned to the spinal anesthesia alone (S, N = 35), the SD anesthesia (SD, N = 33), or the SI anesthesia (SI, N = 38) group by randomization using computer-generated lists. The numbers of patients in the current studies were simply enrolled and no patients were lost for observation because they all need anesthesiologists to remove the spinal catheters at 24 h after the anesthesia. In all, 1.5 ml CSF was obtained before the administration of local anesthetic (2 ml of 1% tetracaine) in the patients in S, SD, and SI anesthesia groups. The participants in S anesthesia group did not receive sedative medicines. General anesthesia was induced with 1.5–2.0 mg/kg propofol (AstraZeneca, Beijing, China) to the participants in the SD and SI anesthesia groups. The patients were not intubated, rather a laryngeal mask airway (a device to help patient to breath) was inserted in the mouth of participants who had general anesthesia. Desflurane (Baxter, Deerfield, IL) or isoflurane (Baxter) was administered to the participants in SD or SI anesthesia groups from an anesthesia machine through the laryngeal mask airway, respectively. Standard anesthesia care was applied, including routine monitors with electrocardiogram, blood pressure, and oxygen saturation. All of the participants had Bispectral Index monitor to determine the relative depth of the general anesthesia. The desflurane or isoflurane concentration was adjusted to maintain the bispectral index value between 50 and 60. Desflurane or isoflurane anesthesia was given for 2 h and no other anesthetics was administered. The CSF was obtained again at 2 and 24 h after the start of the S, SD, or SI anesthesia through the inserted spinal catheter. The spinal catheter was removed immediately following the CSF withdrawal at 24 h after the anesthesia. There was no significant difference in pain scale or opiate usage in patients among S, SD, and SI anesthesia groups after the surgery. We only included patients who had the first surgery of the day in our studies. Thus, the times at which CSF was collected (baseline, 2 h, and 24 h after the anesthesia and surgery) were similar among the patients, which avoids the confounding influence from the normal hour-to-hour variation in CSF $A\beta$ levels as described in previous studies.³²

CSF $A\beta$ and Tau Measurement

$A\beta$ levels (including $A\beta_{40}$ and $A\beta_{42}$) in the CSF were measured using enzyme-linked immunosorbent assay kits (Wako, Richmond, VA) as described by Zhang *et al.*³¹ with modification. Briefly, the collected CSF was stored in a -80° freezer and was thawed before measurement. The sample was centrifuged at 3,000 rpm for 5 min, and then was diluted to 5- and 50-fold for the $A\beta_{42}$ and $A\beta_{40}$ measurements,

respectively, with artificial CSF. Then, 96-well plates were coated with human monoclonal antibodies specific to A β 40 (BA27) or A β 42 (BC05). Following the blocking with Block Ace, wells were incubated overnight at 4°C with test samples of CSF, and then an anti-A β (α -A β -HR1) conjugated to horseradish peroxidase was added. The plates were developed with 3,3',5,5' tetramethylbenzidine reagent, and the well absorbance was measured at 450 nm. The A β levels in the test samples were determined by comparison with the signals from unconditioned artificial CSF spiked with known quantities of A β 40 and A β 42. The levels of tau in human CSF were determined with an enzyme-linked immunosorbent assay kit (Invitrogen, San Francisco, CA). Specifically, a monoclonal antibody specific for human tau was coated onto the wells of the provided microtiter strips. Standards of known human tau content, CSF, and control specimens were pipetted into these wells overnight. After washing, a rabbit polyclonal antibody specific for human tau was added. During the second incubation, this antibody bound to the immobilized human tau captured during the first incubation. After removal of the excess of the second antibody, a horseradish peroxidase-labeled anti-rabbit antibody was added. This bound to the rabbit polyclonal antibody to complete the four-member sandwich. After a third incubation and washing to remove the excess anti-rabbit antibody, a substrate solution was added, which was acted upon by the bound enzyme to produce a change in color. The intensity of this colored product was directly proportional to the concentration of human tau present in the CSF. The range of detection for total tau was 0–20,000 pg/ml. All of the assays were performed in triplicates and the average from the triplicates was obtained. The effects of S, SD, or SI anesthesia on the human CSF A β (and tau) levels were presented as raw data.

Statistics

Since the *in vitro* and the animal studies have suggested that isoflurane, but not desflurane, can increase A β levels, the primary outcomes in our human relevant studies were A β 40 and A β 42 levels in human CSF. We also measured tau levels in the CSF. The data were presented as mean \pm standard deviation. We applied one two-way repeated measures ANOVA to compare the differences in changes in the outcome (*e.g.*, A β 40) among the S, SD, and SI anesthesia groups. Bonferroni adjustment was applied to account for multiple comparisons. The one-way ANOVA with *post hoc* Bonferroni test was used to compare the difference in bispectral index value and admission characteristics (*e.g.*, age and heart rate) in the participants of S, SD, and SI anesthesia groups. We initially enrolled 17, 15, and 20 participants in the S, SD, and SI anesthesia groups, respectively. An article based on the findings from the study was reviewed and the study was determined to be underpowered. In response to reviewers' criticism, we performed power analysis based on the results from the original studies and found that at least

33 participants in each arm would be needed to achieve 80% power to determine the difference in A β and tau values between each arm. We therefore performed additional studies and increased 18 participants in each group to the sample size of 35, 33, and 38 participants in the S, SD, and SI groups, respectively. To control the overall type 1 error in the two phases of analysis, we decided to use a conservative threshold of $P < 0.025$ for statistical significance in the analysis of the data obtained from the combination of both original and second set of participants. We used SAS software (Cary, NC) to analyze the data.

Results

A total of 106 participants were included in the studies. Among them, 55 participants were men and 51 were women. The age of these participants were between 64 and 73 yrs. These participants were randomly assigned into three groups (S, SD, and SI). There was no significant difference in age, height, body weight, education, sex ratio, American Society of Anesthesiologists classification, length of anesthesia, length and type of surgery, estimated blood loss, heart rate, or mean arterial pressure in the participants among the S, SD, and SI anesthesia groups (table 1). The study procedures were well tolerated and there were no adverse events associated with anesthesia in any of the participants.

The preoperative baseline A β 40 and A β 42 levels among the S, SD, and SI anesthesia groups were not significantly different (table 2), A β 40: $F = 2.37$, $P = 0.10$; and A β 42: $F = 1.68$, $P = 0.19$. The concentration of isoflurane (between 1 and 1.5%) or desflurane (between 6 and 9%) was adjusted to maintain a bispectral index value between 50 and 60. These bispectral index values indicate a satisfactory level of anesthesia. Bispectral index value was similar between SD ($57\% \pm 7$) and SI (59 ± 6) anesthesia groups, but both of them were lower than that of the S ($96\% \pm 4$) anesthesia group. There was no significant difference between baseline and postoperative 2- or 24-h human CSF levels of A β 40, A β 42, and tau in all of the participants (grouping effects) (data not shown). These data suggested that the surgery might not significantly alter the CSF A β and tau levels in the current studies.

We first assessed the effects of isoflurane and desflurane on CSF A β 40 levels at 2 and 24 h after the surgery under anesthesia. The mean of preoperative baseline and the 2-h postoperative CSF A β 40 levels in the S, SD, and SI anesthesia groups were 11.59 ± 2.73 and 11.08 ± 2.59 , 12.35 ± 2.86 and 11.28 ± 2.67 , and 10.90 ± 2.56 and 10.23 ± 3.25 ng/ml, respectively (fig. 1A). Two-way repeated measures ANOVA showed no significant difference in human CSF A β 40 levels between SI or SD with S at 2 h after the surgery under anesthesia ($F = 0.66$, $P = 0.52$, N.S.). The difference between the 2-h postoperative and the preoperative baseline CSF A β 40 levels following SI ($F = 0.12$, $P = 0.73$, N.S.) or SD ($F = 1.26$, $P = 0.26$, N.S.) was not statistically different

Table 1. Admission Characteristics of Study Population

	S (N = 35)	SD (N = 33)	SI (N = 38)
Age, yr	69.2 ± 2.1	68.8 ± 2.5	69.3 ± 3.1
Height, cm	168.2 ± 7.9	167.6 ± 8.1	168.3 ± 8.3
Body weight, kg	67.8 ± 6.1	67.3 ± 7.1	66.9 ± 7.9
Education, yr	8.4 ± 2.3	8.1 ± 2.7	8.2 ± 2.5
Sex (M/F)	19/16	16/17	20/18
ASA I	12	11	13
ASA II	23	22	25
Length of anesthesia, min	172.5 ± 22.9	173.03 ± 23.1	171.8 ± 21.9
Length of surgery, min	133.3 ± 22.6	132.8 ± 23.1	134.2 ± 22.8
Orthopedic surgery	25	23	26
Abdominal surgery	10	10	12
Estimated blood loss, ml	190.8 ± 161.2	182.3 ± 162.8	174.9 ± 158.7
Heart rate	74.7 ± 7.4	73.1 ± 7.0	72.7 ± 5.4
Mean arterial pressure	78.5 ± 7.2	76.2 ± 4.3	76.1 ± 4.4

The age, height, body weight, education, sex ratio, ASA classification, length of anesthesia, length and type of surgery, and estimated blood loss are similar between the surgery under S (N = 35), SD (N = 33), and SI (N = 38) anesthesia group.

ASA = American Society of Anesthesiologists; S = spinal; SD = spinal plus desflurane; SI = spinal plus isoflurane.

from that following S anesthesia. These findings suggest that isoflurane or desflurane may not alter the CSF A β 40 levels at 2 h after the surgery under the anesthesia.

Given the findings that isoflurane increases A β levels in brain tissues of mice at 24, but not 6, h after the anesthesia,²⁶ next, we investigated the effects of isoflurane and desflurane on CSF A β 40 levels at a longer time (24 h) after the surgery under anesthesia. The mean of preoperative baseline and the 24-h postoperative CSF A β 40 levels in the S, SD, and SI anesthesia groups were 11.59 ± 2.73 and 11.77 ± 2.81, 12.35 ± 2.86 and 11.81 ± 2.78, and 10.90 ± 2.56 and 12.41 ± 2.82 ng/ml, respectively (fig. 1B). Two-way repeated measures ANOVA showed that there was a statistically significant difference in CSF A β 40 levels among S, SD, and SI at 24 h after the surgery under anesthesia (F = 4.25, * P = 0.02). *Post hoc* Bonferroni test showed that the difference between the 24-h postoperative and the preoperative baseline CSF A β 40 levels following SI anesthesia (1.51 ± 2.76 ng/ml) was higher than that following S anesthesia (0.18 ± 1.89 ng/ml) (fig. 1B) (F = 7.62, ** P = 0.006). In contrast, *post hoc* Bonferroni test showed that the difference in CSF A β 40 levels following SD anesthesia was not statistically different from that following S anesthesia (F = 0.34, P = 0.56, N.S.). Given that there was no significant difference in baseline CSF A β 40 levels among S, SD, and SI groups, these results suggest that isoflurane (SI anesthesia), but

not desflurane anesthesia (SD anesthesia), may be associated with increases in human CSF A β 40 levels at 24 h after the surgery under anesthesia.

Next, we assessed the effects of S, SD, and SI anesthesia on CSF A β 42 levels at 2 and 24 h after the surgery under anesthesia. The mean of preoperative baseline and the 2-h postoperative CSF A β 42 levels in the S, SD, and SI anesthesia groups were 0.43 ± 0.10 and 0.44 ± 0.07, 0.39 ± 0.09 and 0.35 ± 0.08, and 0.39 ± 0.11 and 0.37 ± 0.12 ng/ml, respectively (fig. 2A). Two-way repeated measures ANOVA showed that there was a statistically significant difference in CSF A β 42 levels among S, SD, and SI groups at 2 h after the surgery under anesthesia (F = 4.27, * P = 0.01). *Post hoc* Bonferroni test showed that the difference between the 2-h postoperative and the preoperative baseline CSF A β 42 levels following SD anesthesia (−0.04 ± 0.05 ng/ml) was lower than that following S anesthesia (0.01 ± 0.07 ng/ml) (fig. 2A) (F = 8.41, ** P = 0.004). In contrast, *post hoc* Bonferroni test showed that the difference in CSF A β 42 levels following SI anesthesia was not statistically different from that following S anesthesia (fig. 2A) (F = 3.17, P = 0.08, N.S.). Given that there was no significant difference in baseline CSF A β 42 levels among S, SD, and SI groups, these results suggest that isoflurane (SI anesthesia) may not significantly alter CSF A β 42 levels at 2 h after the surgery under anesthesia.

Table 2. Preoperative Baseline CSF A β Levels between S, SD, and SI Anesthesia Groups

	S (N = 35)	SD (N = 33)	SI (N = 38)	F Values	P Values
A β 40, ng/ml	11.59 ± 2.73	12.35 ± 2.90	10.90 ± 2.6	2.37	0.10
A β 42, ng/ml	0.43 ± 0.95	0.39 ± 0.09	0.39 ± 0.11	1.68	0.19

The preoperative baseline A β 40 and A β 42 levels are not significantly different among surgery under S (N = 35), SD (N = 33), and SI (N = 38) anesthesia groups.

A β = β -amyloid protein; CSF = cerebrospinal fluid; S = spinal; SD = spinal plus desflurane; SI = spinal plus isoflurane.

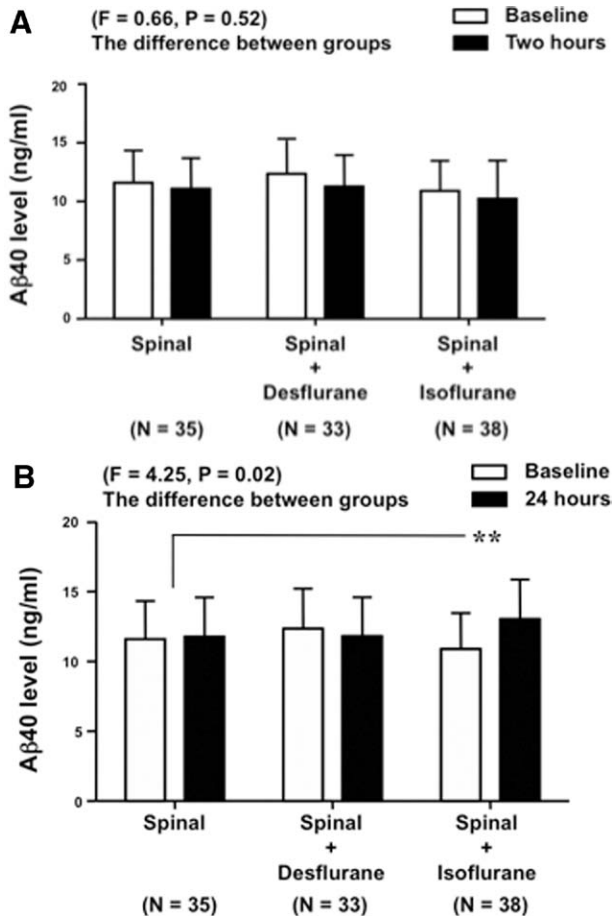


Fig. 1. Surgery under isoflurane anesthesia is associated with increases in human cerebrospinal fluid (CSF) β -amyloid protein (A β)40 levels 24 h after the anesthesia. The preoperative baseline, the 2- and 24-h postoperative human CSF A β 40 levels following spinal (S), spinal plus desflurane (SD), and spinal plus isoflurane (SI) anesthesia are presented. **A**, The differences between the 2-h postoperative and preoperative baseline human CSF A β 40 levels are not significantly different among the S, SD, or SI anesthesia group ($F = 0.66, P = 0.52, N.S.$). **B**, The differences between the 24-h postoperative and the preoperative baseline human CSF A β 40 levels are significantly different among the S, SD, or SI anesthesia group ($F = 4.25, * P = 0.02$). Specifically, the difference between the 24-h postoperative and the preoperative baseline human CSF A β 40 levels following SI anesthesia is positively greater than that following S anesthesia, $F = 7.62, ** P = 0.006$.

Interestingly, desflurane (SD anesthesia) may be associated with decreases in the CSF A β 42 levels at 2 h after the surgery under anesthesia.

Finally, we found that neither desflurane nor isoflurane significantly altered the CSF A β 42 levels 24 h after the surgery under anesthesia: $F = 2.21, P = 0.11, N.S.$ (fig. 2B). The mean of preoperative baseline and the 24-h postoperative CSF A β 42 levels in the S, SD, and SI anesthesia groups were 0.43 ± 0.10 and 0.43 ± 0.10 , 0.39 ± 0.09 and 0.36 ± 0.09 , and 0.39 ± 0.11 and 0.39 ± 0.12 ng/ml, respectively (fig. 2B). These findings suggest that isoflurane, but not desflurane,

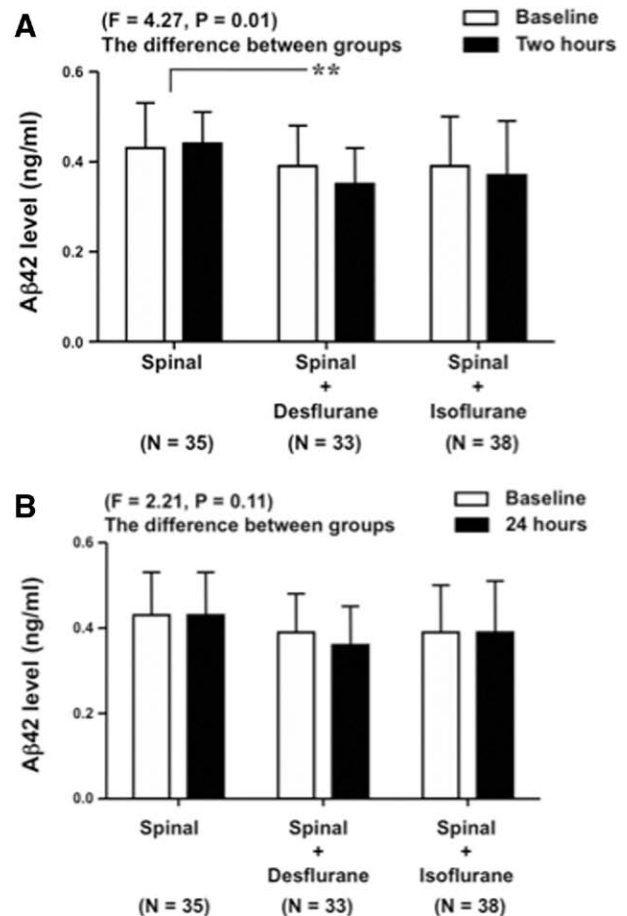


Fig. 2. Surgery under desflurane anesthesia is associated with decreases in human cerebrospinal fluid (CSF) β -amyloid protein (A β)42 levels 2 h after the anesthesia. The preoperative baseline, the 2- and 24-h postoperative human CSF A β 42 levels following spinal (S), spinal plus desflurane (SD), and spinal plus isoflurane (SI) anesthesia are presented. **A**, The differences between the 2-h postoperative and the preoperative baseline human CSF A β 42 levels are significantly different among the S, SD, or SI anesthesia group ($F = 4.27, P = 0.01$). Specifically, the difference between the 2-h postoperative and the preoperative baseline human CSF A β 42 levels following SD anesthesia is negatively greater than that following S anesthesia ($F = 8.41, ** P = 0.004$). **B**, The differences between the 24-h postoperative and the preoperative baseline human CSF A β 42 levels are not significantly different among the S, SD, or SI anesthesia group ($F = 2.21, P = 0.11$).

may be associated with an increase in human CSF A β levels at a longer, but not shorter, time after the surgery under anesthesia, which is consistent with the results from the animal studies that isoflurane increases A β levels in mouse brain tissue at 24, but not 6 or 12, h after the anesthesia.²⁶

Given that tau also plays roles in AD neuropathogenesis and that the ratio of A β 42 to tau in CSF has been suggested as a biomarker of AD,^{21,23} we also assessed the effects of S, SD, and SI anesthesia on the levels of total tau, the ratio of A β 40 or A β 42 to tau in human CSF. The preoperative baseline tau levels among the S, SD, and SI anesthesia groups

Table 3. Neither Ioflurane nor Desflurane Affects Human CSF Tau Levels at 2 or 24 Hours after the Anesthesia

	S (N = 35)	SD (N = 33)	SI (N = 38)	F Values	P Values
Tau (ng/ml)—baseline	0.48 ± 0.13	0.49 ± 0.17	0.43 ± 0.12	1.67	0.19
Tau (ng/ml) —2 h	0.45 ± 0.17	0.48 ± 0.17	0.41 ± 0.13	0.58	0.56
Tau (ng/ml) —24 h	0.51 ± 0.15	0.49 ± 0.18	0.44 ± 0.12	1.03	0.36

The baseline tau protein levels in CSF are not significantly different among the surgery under S (N = 35), SD (N = 33), and SI (N = 38) anesthesia groups. The differences between the 2- or 24-h postoperative and the preoperative baseline human CSF tau levels are not significantly different among the S, SD, or SI anesthesia groups.

CSF = cerebrospinal fluid; S = spinal; SD = spinal plus desflurane; SI = spinal plus isoflurane.

were not significantly different ($F = 1.67$, $P = 0.19$, table 3). We then found that SD and SI anesthesia did not increase the levels of total tau in human CSF compared to S anesthesia at 2 ($F = 0.58$, $P = 0.56$, N.S.) or 24 h ($F = 1.03$, $P = 0.36$, N.S.) after the surgery under anesthesia (table 3).

One two-way repeated measures ANOVA showed that there was no statistically significant difference in the ratio of A β 40 to tau among S, SD, and SI at 2 ($F = 3.48$, $P = 0.04$, N.S.) or 24 h ($F = 2.43$, $P = 0.09$, N.S.) after the anesthesia (table 4). However, the one two-way repeated measures ANOVA illustrated that there was a statistically significant difference in the ratio of A β 42 to tau among S, SD, and SI groups at 2 h after the surgery under anesthesia, $F = 6.83$, ** $P = 0.001$ (table 5). *Post hoc* Bonferroni test suggested that the A β 42 to tau ratio following SD was lower than that following S: 0.84 ± 0.37 versus 1.10 ± 0.41 , $F = 13.28$, ** $P = 0.0003$, at 2 h after the surgery under anesthesia.

Finally, one two-way repeated measures ANOVA showed that there was no statistically significant difference in the ratio of A β 42 to tau among S, SD, and SI groups at 24 h after the surgery under anesthesia, $F = 0.13$, $P = 0.88$, N.S. (table 5).

Discussion

We have found that isoflurane (SI anesthesia) might be associated with higher levels of human CSF A β 40 levels at 24 h after the surgery under anesthesia. Isoflurane may increase neither CSF A β 40 levels at 2 h after the surgery under anesthesia nor CSF A β 42 levels at 2 or 24 h after the surgery under anesthesia. These findings are consistent with the *in vitro* and animal studies that isoflurane can increase A β levels,^{25,26} and particularly the findings that isoflurane increases A β levels in mouse brain tissue at 24, but not 6 or 12, h after anesthesia.²⁶

In contrast to isoflurane, desflurane (SD anesthesia) may not be associated with increases in CSF A β 40 or A β 42 levels. Rather, desflurane is associated with a decrease in CSF A β 42 levels at 2 h after the surgery under anesthesia. These results are consistent with the findings that desflurane does not increase A β levels *in vitro*.³¹

Given that it is difficult to directly measure human brain A β levels following surgery under anesthesia, we used the human CSF A β levels as an alternative approach. The findings that isoflurane anesthesia may be associated with an increase in CSF A β levels suggest that isoflurane may increase brain A β levels. Many studies have suggested that low CSF A β level, at nonintervention status, represents high brain A β amounts, owing to the sequestration of A β into brain amyloid plaques,³³ and is a biomarker for AD and AD progress.^{23,24} However, the CSF A β levels in the current studies represent acute changes following the intervention of surgery under anesthesia. Therefore, it could be postulated that isoflurane may increase human brain A β levels at 24 h following the surgery under anesthesia, which likely leak into the CSF, leading to the elevation of human CSF A β levels.

We did not observe that isoflurane increased the levels of total tau protein in human CSF in the current studies. These findings are consistent with the observations that isoflurane does not increase the total tau levels.^{30,34} However, a recent study has illustrated that the intravenous anesthetic propofol³⁵ and isoflurane³⁰ can induce tau phosphorylation in brain tissues of mice. Future human relevance studies may include assessing the effects of propofol anesthesia and isoflurane anesthesia on the levels of phosphorylated tau in human CSF.

Previous animal studies showed that isoflurane induced caspase-3 activation and increased levels of β -site amyloid precursor protein–cleaving enzyme (BACE, the enzyme for the generation of A β) at 6 h after the anesthesia, but enhanced

Table 4. Neither Desflurane nor Isoflurane Alters A β 40 to Tau Ratio in Human CSF at 2 Hours after the Anesthesia

	S (N = 35)	SD (N = 33)	SI (N = 38)	F Values	P Values
A β 40/Tau—2 h	27.45 ± 9.94	25.88 ± 9.63	27.10 ± 11.14	3.48	0.04
A β 40/Tau—24 h	24.75 ± 8.53	29.07 ± 9.87	30.11 ± 10.25	2.43	0.09

The A β 40 to tau ratio in human CSF is not significantly different among the surgery under S (N = 35), SD (N = 33), and SI (N = 38) anesthesia groups at 2 or 24 h after the surgery under anesthesia.

A β = β -amyloid protein; CSF = cerebrospinal fluid; S = spinal; SD = spinal plus desflurane; SI = spinal plus isoflurane.

Table 5. Desflurane, but Not Isoflurane, Reduces Human CSF A β 42 to Tau Ratio at 2, but Not 24, Hours after the Anesthesia

	S (N = 35)	SD (N = 33)	SI (N = 38)	F Values	P Values
A β 42/Tau—2 h	1.10 \pm 0.41	0.83 \pm 0.33**	1.02 \pm 0.52	6.83	0.001
A β 42/Tau—24 h	0.91 \pm 0.36	0.84 \pm 0.37	0.97 \pm 0.46	0.13	0.88

The A β 42 to tau ratio in human CSF is lower following the surgery under SD (N = 33), but not SI (N = 38), compared to S (N = 35) anesthesia group at 2, but not 24, h after the surgery under anesthesia, F = 13.28, ** P = 0.0003.

A β = β -amyloid protein; CSF = cerebrospinal fluid; S = spinal; SD = spinal plus desflurane; SI = spinal plus isoflurane.

A β levels at 24, but not 6 or 12, h after the anesthesia in the brain tissues of mice.²⁶ These results suggest that isoflurane may induce caspase activation and apoptosis, which in turn increase BACE activity, leading to A β generation in a time-dependent manner. This hypothesis is further supported by the *in vitro* mechanistic studies that isoflurane may induce a cycle of caspase activation/apoptosis and increases in BACE levels and A β generation.³⁶ The findings from the current human studies that isoflurane might increase A β levels at longer, but not shorter, time after the surgery under anesthesia also suggest that isoflurane may induce a time-dependent increase in A β levels potentially *via* this caspase/apoptosis–BACE–A β cascade.

Alternatively, isoflurane may increase the A β levels *via* noncaspase/apoptosis–BACE–A β cascade. Wan *et al.*³⁷ showed that surgery under anesthesia might induce neuroinflammation, leading to A β accumulation in brain tissues of mice. Isoflurane has been shown to increase the levels of pro-inflammatory cytokines in mouse brain tissues.³⁸ We therefore postulate that isoflurane increases A β levels through the isoflurane-induced neuroinflammation and we will test this hypothesis in the future studies.

Interestingly, we found that desflurane might decrease the A β 42 levels in human CSF 2 h after the surgery under SD anesthesia (fig. 2A). Furthermore, at 2 h after the surgery under anesthesia, both isoflurane (SI) and desflurane (SD) seem to decrease A β 40 and A β 42 levels, even though some of the decreases did not reach statistically significant levels (figs. 1A and 2A). The underlying mechanisms of these findings remain to be determined. It has been shown that reduction in brain synaptic activity^{39,40} and brain function⁴¹ leads to decreases in A β levels. The findings that human CSF A β levels decrease at 2, but not at 24, h following the surgery under isoflurane or desflurane anesthesia suggest that the anesthesia-induced reduction in brain function, as evidenced by the reduction in the bispectral index value, may lead to the decreases in the A β levels. Future research should include time course studies of the effects of isoflurane, desflurane, and other anesthetics (*e.g.*, intravenous anesthetic propofol) on human CSF A β levels to further test this hypothesis. We may determine the CSF A β levels every hour between 2 and 24 h postoperatively.

It is interesting that isoflurane only increased the levels of A β 40, but not A β 42, in the human CSF in the current

experiments. Although A β 42 seems more pathogenic than A β 40,⁴² several studies have shown that A β 40 is neurotoxic and can induce caspase activation and apoptosis.^{16–18} In addition, A β 40 has been shown to induce impairment of synaptic plasticity and memory function.^{19,20} Finally, increased A β 40 levels in animal brains can occur after stroke and brain ischemia⁴³ and following energy inhibition.⁴⁴ Thus, the acute increase in A β 40 may also cause neurotoxicity, impair synapse function, and induce learning and memory impairment.

In the present pilot studies, we have established a system to determine the effects of anesthesia on A β and tau levels in human CSF and have demonstrated the *in vivo* relevance in humans of our *in vitro* and animal findings that isoflurane, but not desflurane, may increase A β levels. These results suggest that isoflurane and desflurane may affect human CSF A β levels differently. Specifically, isoflurane may increase human CSF A β 40 levels at 24 h after the surgery under anesthesia, and desflurane may decrease A β 42 levels at 2 h after the surgery under anesthesia. These findings could have a significant impact on clinical practice of anesthesia and suggest that further studies to determine the effects of anesthesia on AD biomarkers are warranted.

There are two pioneer studies in investigating the effects of anesthesia and surgery on human CSF biomarkers associated with AD. Palotas *et al.*⁴⁵ determined the effects of coronary artery bypass under general anesthesia on human CSF level of A β 42, Tau, and S100 β and found that A β 42, Tau, and S100 β significantly increased at 1 week after the surgery. However, 6 months after the surgery, the A β 42 levels in the human CSF significantly decreased although the human CSF levels of tau and S100 β still increased.⁴⁵ In another study, Tang *et al.*⁴⁶ found that the idiopathic nasal CSF leak correction under anesthesia with propofol, remifentanyl, or sevoflurane increased the human CSF levels of tau and proinflammatory cytokines, but not A β 42, 48 h after the surgery. Our current human studies are different from these two studies in postoperative time intervals (2 and 24 h *vs.* 48 h, 1 week, and 6 months), anesthesia (isoflurane or desflurane *vs.* propofol, remifentanyl, or sevoflurane), surgery (orthopedic or abdominal surgery *vs.* coronary artery bypass or idiopathic nasal CSF leak correction), and measurement (A β 40 *vs.* A β 42). Future studies to determine whether different anesthesia, surgery, and postoperative

time intervals may have different effects on specific human CSF biomarkers associated with AD are warranted.

There are several caveats in the current studies. First, we did not plan to perform the interim analysis before the proposed studies. We recruited a second set of subjects when we realized that we could have type 1 error in the analysis of the data from the original set of subjects. To control the overall type 1 error rate to no more than 0.05, we chose to use $P < 0.025$ as the criterion for statistical significance for each analysis of the data obtained from the combination of both the original and the second set of participants. Second, the effects of isoflurane on the elevation of human CSF A β levels are moderate (smaller than diurnal variation of human CSF A β levels); therefore, it is uncertain that the isoflurane- or desflurane-induced changes in brain or CSF A β levels account for the isoflurane- or desflurane-induced changes in cognitive function. Future studies will determine the potential association between the isoflurane-induced changes in cognitive function and in brain, CSF, or plasma A β levels, which may illustrate the role of anesthetic isoflurane in the neuropathogenesis of AD, as well as in the decline of postoperative cognitive function.

In conclusion, our studies have established a system to investigate the effects of anesthesia on human biomarkers associated with AD and cognitive dysfunction. The findings from these studies have suggested that surgery under isoflurane anesthesia may be associated with increases, but surgery under desflurane anesthesia may be associated with decreases in human CSF A β levels. These results will promote more human biomarker studies to either rule in or rule out the role of anesthesia in the neuropathogenesis of AD and postoperative cognitive dysfunction.

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