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The Effect of Propofol Versus Isoflurane Anesthesia on Human Cerebrospinal Fluid Markers of Alzheimer's Disease: Results of a Randomized Trial

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Abstract

Background—Preclinical studies have found differential effects of isoflurane and propofol on the Alzheimer's disease (AD)-associated markers tau, phosphorylated tau (p-tau) and amyloid- β (A β).

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SUPPLEMENTARY MATERIAL

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Objective—We asked whether isoflurane and propofol have differential effects on the tau/A β ratio (the primary outcome), and individual AD biomarkers. We also examined whether genetic/intraoperative factors influenced perioperative changes in AD biomarkers.

Methods—Patients undergoing neurosurgical/otolaryngology procedures requiring lumbar cerebrospinal fluid (CSF) drain placement were prospectively randomized to receive isoflurane ($n = 21$) or propofol ($n = 18$) for anesthetic maintenance. We measured perioperative CSF sample AD markers, performed genotyping assays, and examined intraoperative data from the electronic anesthesia record. A repeated measures ANOVA was used to examine changes in AD markers by anesthetic type over time.

Results—The CSF tau/A β ratio did not differ between isoflurane- versus propofol-treated patients ($p = 1.000$). CSF tau/A β ratio and tau levels increased 10 and 24h after drain placement ($p = 2.002 \times 10^{-6}$ and $p = 1.985 \times 10^{-6}$, respectively), mean CSF p-tau levels decreased ($p = 0.005$), and A β levels did not change ($p = 0.152$). There was no interaction between anesthetic treatment and time for any of these biomarkers. None of the examined genetic polymorphisms, including *ApoE4*, were associated with tau increase ($n = 9$ polymorphisms, $p > 0.05$ for all associations).

Conclusion—Neurosurgery/otolaryngology procedures are associated with an increase in the CSF tau/A β ratio, and this increase was not influenced by anesthetic type. The increased CSF tau/A β ratio was largely driven by increases in tau levels. Futurework should determine the functional/prognostic significance of these perioperative CSF tau elevations.

Keywords

Amyloid-beta; anesthesia; cerebrospinal fluid; isoflurane; propofol; surgery; tau protein

INTRODUCTION

The most prevalent type of dementia is Alzheimer's disease (AD). In AD, amyloid- β (A β) monomers oligomerize into insoluble plaques (thus decreasing monomeric cerebrospinal fluid (CSF) A β levels), and the soluble microtubule stabilizing protein tau deposits into insoluble aggregates (i.e., "tau tangles") [1]. The clinical onset of AD dementia is preceded by gradual increases in CSF total tau levels and gradual decreases in CSF A β levels that occur over decades [1], which together produce an increase in the CSF tau/A β ratio [2]. However, it is unclear what factors initiate or promote these pathologic processes. A small percentage of AD cases are due to dominant genetic mutations, while up to 50% of AD cases may be caused by environmental exposures [3]. Preclinical studies suggest that anesthesia and/or surgery could be environmental exposures that increase AD risk by causing increased A β levels [4], A β oligomerization [5], increased tau levels [6, 7], tau phosphorylation [8], neuroinflammation [9], and neuroapoptosis [10] (reviewed in [11]). If anesthesia and/or surgery accelerate AD pathogenesis in patients, this could potentially contribute to delirium, postoperative cognitive dysfunction, or longer-term cognitive decline, such as the eventual development of AD. Two small human studies have shown that CSF total tau levels increase after anesthesia and surgery [6, 7], demonstrating that some of these preclinical findings translate to humans.

Laboratory studies also suggest that inhaled anesthetic agents may promote AD pathogenesis to a greater extent than propofol [12, 13]. In fact, propofol blocks isoflurane-induced apoptosis and A β oligomerization in cell culture and in the mouse brain [12, 13]. If these findings extend to humans, they could be clinically important because specific anesthetic agents could be utilized to help decrease perioperative effects on AD biomarkers and pathogenesis, particularly in patients at higher risk of developing AD. Tang et al. examined CSF AD biomarkers in 11 patients who received sevoflurane or propofol, but this trial was non-randomized [7] and did not examine CSF AD biomarker differences between anesthetic groups. To date, no human randomized clinical trial has examined whether propofol versus isoflurane have differential effects on human CSF AD biomarkers after surgery. Thus, we examined this question in a pilot randomized trial. We chose the CSF tau/A β ratio between patients treated with isoflurane versus propofol as our primary outcome measure, since the CSF tau/A β ratio has high sensitivity and specificity for the diagnosis of AD [2]. Because genetic factors (such as ApoE4 [14]) and vascular disease (such as hypertension, diabetes and hypercholesterolemia [3]) contribute to AD risk and to changes in AD biomarkers, we also examined whether genetic and/or intraoperative parameters would predict the extent of postoperative AD biomarker changes.

MATERIALS AND METHODS

Study protocol

This trial was approved by the Duke University Institutional Review Board, and registered with <http://www.clinicaltrials.gov> (identifier NCT01640275) on June 20, 2012, by Miles Berger, the study PI. Patients scheduled for neurosurgical or otolaryngology procedures including lumbar CSF drain placement were prospectively enrolled in this trial. We excluded patients who were 1) not able to give informed consent, 2) < 18 years old, 3) pregnant, 4) imprisoned, or 5) who had a personal or family history of malignant hyperthermia or other medical contraindication to receiving isoflurane or propofol. There was no exclusion for cognitive impairment (such as clinical AD, mild cognitive impairment, or any other form of cognitive impairment), aside from the requirement that patients be capable of giving informed consent. Patients were randomized to receive either isoflurane or propofol for anesthetic maintenance; all patients received propofol for anesthetic induction. Randomization was performed according to a computer-generated random number list. Patients were blinded to their anesthetic group assignment. Anesthesia providers were instructed to titrate propofol or isoflurane dosage (depending on the patient's randomization assignment) to maintain a bispectral index (BIS) range of 40–60 in both study arms. There were no other study requirements regarding anesthetic technique or the dosing of opioids, paralytics, or steroids. There was no exclusion for surgical cases that included neuromonitoring, such as brainstem auditory evoked responses. A member of the surgical team percutaneously introduced a subdural Silastic® catheter at the L4–5 or L5–S1 interspace, and connected it to an external CSF drain (AccuDrain INS-8400; Integra Neurosciences, Plainsboro, NJ). The Silastic® catheter and CSF drain were placed within one hour after induction of general anesthesia in all cases.

CSF sampling

At the time of drain placement (0) and 10 and 24 h later, 10 ml of fresh CSF was aseptically collected from the CSF drain and placed on ice in a 15 ml conical tube (VWR; Radnor, PA). CSF samples were separated into 1-ml aliquots, using low-binding 1000- μ l pipette tips (Genesee; San Diego, CA), placed in Sarstedt 1.5-ml polypropylene microcentrifuge tubes (VWR; Radnor, PA), and stored at -80°C .

AD biomarkers

We submitted all CSF sample aliquots on dry ice to the University of Pennsylvania Biomarker Core Research Laboratory, which has previously performed AD biomarker assays on patients from the Alzheimer's Disease Neuroimaging Initiative (ADNI) studies [2, 15]. $\text{A}\beta_{42}$, t-tau, and p-tau181p were measured in each aliquot using the multiplex xMAP Luminex platform (Luminex Corp; Austin, TX) and Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium) immunoassay kit reagents, according the established protocols used in the ADNI 1 and 2 studies [2, 15]. These kits included well-characterized capture monoclonal antibodies specific for $\text{A}\beta_{42}$ (4D7A3), t-tau (AT120), and p-tau181 (AT270, which selectively binds tau that has been phosphorylated at threonine 181), each chemically bonded to unique sets of color-coded beads, and analyte-specific detector antibodies (HT7, 3D6). Calibration curves were produced for each biomarker using aqueous buffered solutions that contained a combination of three biomarkers at concentrations of 56-1,948 pg/mL for recombinant tau, 27-1,574 pg/mL for synthetic $\text{A}\beta_{1-42}$ peptide, and 8-230 pg/mL for synthetic p-tau peptide phosphorylated at threonine 181. All assays were performed in duplicate, by individuals blinded to anesthetic group and time point.

High inter-laboratory variability has been reported in $\text{A}\beta$ assays [16]. This is thought to occur because plastic can bind to $\text{A}\beta_{42}$ peptides and can cause erroneously low measurements, contaminants can interfere with $\text{A}\beta_{42}$ peptide detection, different immunoassays have been utilized to measure $\text{A}\beta$, and there are methodologic differences in how these assays have been performed between labs [16-20]. Based on these concerns, and because the range of $\text{A}\beta_{42}$ measurements in patients with AD, mild cognitive impairment (MCI), and healthy controls ranged from 80 to 300 pg/ml in the ADNI 1 and 2 studies [2], we excluded $\text{A}\beta_{42}$ measurements from the XMAP ELISA assay that were below 80 pg/ml and set these values to missing. This resulted in the exclusion of 8 $\text{A}\beta_{42}$ measurements, out of 165 $\text{A}\beta_{42}$ measurements overall. A sensitivity analysis showed that excluding these values did not change the lack of an anesthetic group effect on $\text{A}\beta_{42}$ levels or the tau/ $\text{A}\beta_{42}$ ratio.

DNA isolation and SNP analysis

Whole blood (10 ml) was collected from patients at each indicated time point using K_2EDTA vacutainer tubes (Becton Dickinson; Franklin Lakes, NJ) and immediately placed on ice. Samples were then centrifuged at 3,000 RPM for 15 min to separate the red cells and buffy coat layer from plasma. The plasma was divided into 1-ml aliquots, and frozen at -80°C . The remaining blood pellet and buffy coat layer (containing white blood cells) were also frozen at -80°C . To isolate nucleic acids, these frozen blood samples were thawed, inverted 4-6 times to gently mix, and genomic DNA was extracted from a 200 μ l aliquot

using QIAmp DNA Blood Mini Kits from (Qiagen; Gaithersburg, MD). The DNA concentration in each sample was determined using a NanoDropND® –1000 (ThermoFisher Scientific; Grand Island, NY), and DNA was diluted to a concentration of 10 ng/μl in T.E. buffer (10 mM Tris-Cl (pH 7.5), 1mM EDTA). We were unable to obtain DNA from 1 patient. Genotyping was performed by the Duke University DNA Analysis Facility (Durham, NC) using Applied Biosystems TaqMan SNP genotyping assays (10 ng DNA/assay) and an Applied Biosystems 7500 Fast Real-Time PCR system (obtained from ThermoFisher Scientific; Grand Island, NY), according to the manufacturer's instructions. Data analysis was performed using Applied Biosystems 7500 software v2.0.4. The SNP analysis included the following genes with the corresponding 'rs' number: IL6 G/C (rs1800796), IL6R A/C (rs2228145), gp130 A/T (rs1900173), gp130 A/G (rs10940495), MAPT (Tau) exon6 H47Y C/T (rs2258689), MAPT (Tau) exon6 S53P C/T (rs10445337), APOE promoter –219 G/T (rs405509), APOE exon 4 C112R C/T (rs429358), APOE exon 4 C158R C/T (rs7412), and TOMM40C/T (rs157581).

Sample size/Power analysis

The trial was conducted to test the hypothesis that isoflurane and propofol exert differential effects on CSF AD biomarkers in surgical patients, and was based on laboratory studies that found differential effects of these drugs [12, 13]. However, these laboratory studies measured AD biomarkers in whole mouse brain or in cell culture, rather than in CSF. Further, given the inter-species differences between mice and humans, we considered these preclinical data to be insufficient to estimate the likely effect size of any difference in AD biomarkers between patients treated with propofol versus isoflurane. Because there was insufficient preliminary data to perform a power/sample size calculation, we planned to enroll approximately 40 patients in this randomized study to evaluate our primary outcome (the CSF tau/Aβ ratio between anesthetic groups), and to achieve two additional goals. First, we wanted to demonstrate the feasibility of measuring AD biomarkers in the CSF of neurosurgical and otolaryngological patients via a lumbar CSF drain, and second, estimate the effect size of any difference in AD biomarker levels between patients treated with propofol versus isoflurane, data which could be used to calculate the necessary sample size for future studies.

Statistical analysis

Differences in baseline and surgical characteristics between the two treatment groups were assessed with *t* tests or Wilcoxon Rank sum tests for continuous variables, and chi-square or Fisher's exact test for categorical variables in Table 1. Repeated measures 2-way analysis of variance (ANOVA) was used to examine patterns in the levels of CSF tau, p-tau, Aβ, and tau/Aβ and p-tau/Aβ ratios from baseline to 24 h in the two treatment groups. We used residual diagnostics to assess the assumptions of the ANOVA analysis, and performed log transformation of CSF measures where necessary. Beta coefficients, beta coefficient standard errors, and effect sizes (beta coefficient/beta coefficient standard error) for the repeated measures 2-way ANOVA are listed in Table 2. To account for multiple comparisons for each biomarker, we report adjusted *p* values from these models in Table 2, and in the abstract and results section text.

Additionally, 24 h change in tau levels in the full cohort was examined for association with baseline and surgical characteristics via Wilcoxon Rank Sum tests, Kruskal-Wallis Tests or Spearman correlation as appropriate (Table 3). For all analyses, significance was set at $\alpha = 0.05$. SASv9.4 software (Cary, NC) was used for statistical analysis.

Genetic analyses

We examined the association between markers in known cognitive-related candidate genes, and tau changes at 24 h after surgery. Wigginton exact test and chi-square tests implemented in PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink/>) were applied to test Hardy-Weinberg equilibrium (HWE) for each marker. We planned to exclude markers that deviated from HWE ($p < 0.05$) from further analyses. Minor allele frequency (MAF) was obtained for each marker. Because of the low incidence of individuals homozygous for each minor allele, we compared tau changes at 0 to 24 h in patients carrying at least one minor allele (carriers) versus patients without the minor allele (non-carriers) for each marker by a two-sample t -test. This is equivalent to the dominant genetic model. The test of equality of variances was used to determine whether the t -test should be based on pooled variance (equal variance) or unequal variance. Mean and standard deviation (SD) of tau change at 24 h after surgery were computed for non-carrier and carrier groups, respectively (Table 4).

RESULTS

A schematic of study enrollment is shown in Fig. 1. Approximately 80% (i.e., 104/129) of the patients we approached (all of whom were scheduled for cases with lumbar CSF drain placement) agreed to participate in the study. 41 of the 104 patients who consented could not be enrolled because a lumbar CSF drain was not placed (either because the surgeons decided it was unnecessary, or were unable to place it) or did not work properly, resulting in an inability to obtain CSF. We initially planned to draw CSF samples and measure AD biomarkers at 0 and 3 h. However, an interim analysis of 0 and 3 h samples from the first 19 patients showed no significant effect of time, treatment, or interaction between time and treatment for any of the AD biomarkers measured ($p > 0.05$ for all comparisons; Supplementary Figure 1). These data are consistent with an observational time-course study that showed no change in AD markers until 10–24 h after the induction of anesthesia [7]. Based on these findings, we modified our IRB protocol to collect CSF samples at the time of lumbar drain placement and 10 and 24 h later. We did not include data from the patients with 0 and 3 h samples in the final analysis.

Baseline and intraoperative characteristics of the study patients who had samples obtained at 0, 10, and 24 h are presented in Table 1. None of the patients had a diagnosed neurodegenerative disease or MCI. To assess any potential effect of the extent of intracranial surgery itself on CSF AD biomarkers, we separated the surgical cases into 3 groups: 1 = peripheral neurosurgery without deep intracranial work (e.g., trigeminal nerve decompressions or CSF leak repairs), 2 = deep intracranial surgery (e.g., cerebellopontine angle tumor or acoustic neuroma resections), or 3 = miscellaneous cases (e.g., cortical surface meningioma resections). There was no difference in the surgical case mixture between patients in the isoflurane versus propofol groups (Table 1). No statistically

significant differences were found between groups for any of the baseline variables except for total propofol dosage, which was higher (as expected) in the propofol group.

There was no effect of anesthetic group on the CSF tau/A β ratio ($p = 1.000$; our primary outcome, Fig. 2A). An effect of time on the CSF tau/A β ratio was detected ($p = 2.002 \times 10^{-6}$; Fig. 2A), but there was no interaction between time and anesthetic group on the CSF tau/A β ratio ($p = 0.527$). Effect sizes and β coefficients from this repeated measures ANOVA model are presented in Table 2. An effect of anesthetic group on CSF tau levels was not detected ($p = 1.000$). An effect of time on mean CSF tau levels was detected ($p = 1.985 \times 10^{-6}$; Fig. 2B). There was no interaction between time and anesthetic group on CSF tau levels ($p = 1.000$).

There was no effect of anesthetic group on CSF A β levels ($p = 1.000$). An effect of time on CSF A β levels was not detected ($p = 0.152$; Fig. 2C), and there was no interaction between time and anesthetic group on CSF A β levels ($p = 1.000$). An effect of anesthetic group on CSF p-tau levels was not detected ($p = 0.236$). An effect of time on CSF p-tau levels was detected ($p = 0.005$; Fig. 2D). There was no interaction between time and anesthetic group on CSF p-tau levels ($p = 1.000$). There was no effect of anesthetic group on the p-tau/A β ratio ($p = 0.295$), and no effect of time on the CSF p-tau /A β ratio ($p = 0.302$; Fig. 2E). There was no interaction between time and anesthetic group on the p-tau/A β ratio ($p = 1.000$).

To identify factors that might account for inter-patient variation in the perioperative CSF tau increase at 24 h (Fig. 2B), we used rank-based tests to examine 24 h tau change. Of the factors examined in a univariate analysis (Table 3), only intraoperative dexamethasone dosage was associated with the degree of perioperative CSF tau increase at 24 h ($p = 0.04$, unadjusted for multiple comparisons). However, this exploratory *post-hoc* finding did not reach statistical significance after correction for multiple comparisons ($p = 0.76$).

Next, we examined whether there might be a genetic basis for the variation in perioperative CSF tau increases. APOE4 is a well-studied genetic risk factor for sporadic AD (reviewed in [21]), and polymorphisms in genes associated with inflammation (such as IL-6 and its receptor GP130) and mitochondrial dysfunction (such as TOMM40, an outer mitochondrial membrane 40 homolog) have also been implicated in AD [22–25]. Many of these genetic variants also alter CSF AD biomarker levels [26, 27]. Thus, we genotyped patients for these and other related genetic variants, and examined whether these genetic variants were associated with the 24 h CSF tau increase. We were unable to obtain DNA from 1 patient. The remaining 38 patients were genotyped for 9 markers in 5 candidate genes: APOE, GP130, IL6, Tau, and TOMM40. All nine markers met Hardy-Weinberg Equilibrium ($p > 0.05$) and were tested for association with the extent of CSF tau elevation (Table 4). Equality of variance between carrier and non-carrier groups was similar for all markers except rs157581 in TOMM40 ($p = 0.013$). Thus, a 2-sample t test based on unequal variance was applied to rs157581. For the other 8 markers, we used a 2-sample t test with pooled variance. None of these genetic polymorphisms was associated with the 24 h CSF tau level increase (Table 4).

DISCUSSION

The data presented here show no difference in the increase in the CSF tau/A β ratio between patients undergoing neurosurgical/otolaryngologic procedures who were randomized to receive propofol or isoflurane. The CSF tau/A β ratio and tau levels increased over time, but these increases were anesthetic type independent. *In vitro* and animal model studies suggest that anesthetic drugs and surgical stress affect AD pathways (reviewed in [11] and [28]), and that inhaled anesthetics, such as isoflurane, may have a greater effect on these pathways than propofol [12, 13, 29]. However, the patient data presented here do not demonstrate a greater effect of isoflurane than propofol on AD pathways/markers.

Baseline and intraoperative characteristics were well matched between anesthetic groups (Table 1). Propofol dosage was the only characteristic that differed significantly between groups, as expected due to randomization. Although this is a pilot study, it is based on a relatively large data set- we obtained 165 perioperative CSF samples from 63 patients (Fig. 1). There are only four prior reports examining perioperative human CSF AD biomarker changes [6, 7, 30, 31]; this study is larger than all but one of these [31]. This is also the only randomized study comparing the effects of propofol versus inhaled anesthetics on CSF AD biomarkers, a key question based on preclinical studies [12, 13] and the significant pharmacologic differences between propofol and isoflurane. Few perioperative CSF AD biomarker studies have been conducted despite the importance of understanding whether anesthesia and surgery modulate AD pathogenesis [11], because it is difficult to collect CSF samples from human surgical patients. This is the largest human perioperative CSF AD biomarker study performed using standardized laboratory measurements [32] and assays validated by the Alzheimer's Association Global Biomarkers Consortium [33, 34]. This helps ensure the reproducibility of these results, a scientific goal promoted by the National Institute of Health [35]. We measured AD biomarkers in the ADNI biomarker core laboratory according to the ADNI protocol [32]; thus, the measurements reported here can be directly compared to those of the ADNI studies (as described below).

This study provides data about perioperative CSF AD biomarker variance and correlation between measures over time, thus allowing a *post-hoc* power analysis/sample size calculation for future studies on the biomarker measured here. Based on our data and our statistical model's correlation coefficient between repeated measures (0.41), a future study with 18 propofol-treated patients and 21 isoflurane-treated patients with the same repeated measures design would have 80% power (with an $\alpha = 0.01$) to detect a 20% difference in tau levels between groups. A 20% change in CSF tau levels would be clinically significant. In the ADNI study, a 20% increase would move the median CSF tau level found in normal individuals almost halfway toward the median value seen in patients with MCI [2]. This confirms that our study had adequate power for detecting a clinically significant tau difference between groups. To investigate differences in A β , p-tau, the tau/A β ratio, and the p-tau/A β ratio with these same statistical parameters (20% difference, 80% power, $\alpha = 0.01$) and our observed means and correlations, a similar future study would require 45, 73, 72 and 16 patients per anesthetic group, respectively.

The mean tau value among patients at 24 h after surgery was 122 pg/ml (Fig. 2A), which exceeds the mean tau level in patients with MCI, and equals the mean tau level in AD patients in the ADNI study [2]. CSF tau levels > 93 pg/ml have a greater than 90% positive predictive value for AD [2]. We did not detect a difference in A β levels over time (Fig. 2C), although we cannot rule out the possibility that we were underpowered for this outcome as described above. Nonetheless, the tau/A β ratio (Fig. 2A) clearly increased over time due to the rise in CSF tau levels (Fig. 2B). Most earlier studies [6, 7, 36] but not all [31] have reported similar CSF tau and tau/A β ratio elevations after other surgery types.

In AD, CSF tau levels progressively rise prior to the onset of hippocampal atrophy, memory deficits, and clinical dementia [1]. Elevated CSF tau levels have also been detected in boxers [37, 38] and in individuals with traumatic brain injury [39, 40], and may predict long-term cognitive outcomes [39]. Nonetheless, the long-term trajectory of the acute 24 h postoperative changes in CSF tau levels reported here is unknown. It is also unknown whether acute postoperative CSF tau increases correlate with altered short- or long-term neurocognitive function. We are examining these questions in the MADCO-PC study Markers of Alzheimer's Disease and neuroCognitive Outcomes after Perioperative Care <https://www.clinicaltrials.gov/ct2/show/NCT01993836?term=Miles+Berger&rank=1>, which will clarify whether anesthesia and surgery are associated with long term changes in AD biomarkers, and whether such changes are associated with long term cognitive changes.

Interestingly, in a secondary univariate analysis, intraoperative dexamethasone dosage was inversely correlated with the perioperative increase in CSF tau levels. This finding is from a secondary analysis and did not remain statistically significant after multiple comparison correction, but it provides hypothesis-generating preliminary evidence for future research. Glucocorticoids such as dexamethasone have pleiotropic effects on neural gene expression [41, 42], and increase tau gene expression *in vitro* [43]. This suggests that dexamethasone does not decrease CSF tau levels simply by reducing tau gene expression. Instead, perioperative CSF tau elevations may occur via to a neuroinflammatory mechanism (which dexamethasone suppresses), a hypothesis that could be tested in a new trial. Although we found an inverse correlation between intraoperative dexamethasone dosage and the extent of perioperative CSF tau elevation in this secondary analysis, we found no correlation between several genetic polymorphisms that modulate the inflammatory response and the extent of perioperative CSF tau elevations. However, it is possible that we were underpowered to detect this genetic correlation (i.e., a type II error). Larger future studies will be necessary to examine a potential genetic basis for perioperative changes in CSF tau levels.

One caveat to these findings is that all patients underwent direct surgical manipulation of the dura and blood-brain barrier, and many underwent direct intracortical brain surgery. The effects of surgical manipulation of the dura and brain could outweigh the effects of anesthetic type on CSF AD biomarkers, which could explain why no anesthetic group difference was seen here. In this case, there could be differential effects of propofol versus isoflurane on CSF AD biomarkers after other types of surgery. We are currently examining this possibility as a secondary outcome in the MADCO-PC trial.

In conclusion, we found no difference in the CSF tau/A β ratio in patients who were randomized to isoflurane versus propofol anesthesia, although the lack of an anesthetic effect here may be due to a type II error. Future studies would need approximately 20–70 patients per group to have adequate power to detect anesthetic differences in these CSF AD biomarkers. Nonetheless, our data demonstrate that neurosurgery and otolaryngology procedures are associated with 3-fold increases in the CSF tau/A β ratio and total tau levels at 24 h, into the same ranges seen in AD [2]. The clinical and prognostic significance of these perioperative CSF tau/A β ratio and tau level increases is currently unknown and warrants further research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Authors' disclosures available online (<http://j-alz.com/manuscript-disclosures/15-1190r2>).

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Fig. 1.
CONSORT diagram/flow chart of the study population. CSF, cerebrospinal fluid.

Fig. 2. CSF Alzheimer's disease marker levels during and after neurosurgical/otolaryngology cases performed under propofol versus isoflurane maintenance anesthesia. Values = mean $\pm$ 95% confidence interval.

Table 1

	Isoflurane group (N = 21)	Propofol Group (N = 18)	
	Mean (SD) or %	Mean (SD) or %	p value*
<i>Baseline Patient Characteristics</i>			
Age	59.2 (12.6)	50.2 (15.5)	0.0538
Sex (male/ female)	23.8% / 76.2%	5.6% / 94.4%	0.1897
Weight (kg)	82.9 (16.8)	76.3 (15.9)	0.2197
Body mass index	29.2 (5.2)	27.8 (5.9)	0.6121
<i>Baseline Biomarker Variables</i>			
Tau	35.8 (12.7)	48.6 (29.5)	0.4874
P-Tau	25.5 (11.1)	21.2 (7.7)	0.1699
Abeta	280.0 (70.8)	307.5 (84.6)	0.2761
<i>Intraoperative Pharmacologic Variables</i>			
Propofol drip duration (min)	—	264.5 (91.2)	
Average Propofol drip rate (mcg/kg/min)	—	106.8 (16.2)	
Propofol total dosage (mg)	161.7 (76.2)	2120.1 (763.2)	<0.0001
Case Length (min)	370 (109)	324 (103)	0.2423
Anesthetic Duration (min)	370 (119)	312 (97)	0.1631
Average MAC	0.5 (0.1)	—	
Average MAC (hours)	2.8 (1)	—	
Total Remifentanyl Dose (mg)	4.25 (2.41)	3.93 (2.51)	0.5262
Total Fentanyl Dose (mcg)	245.2 (38.4)	230.6 (89.3)	0.2943
Total Hydromorphone Dose (mg)	0.5 (0.5)	0.8 (0.7)	0.1071
Total Dexmedetomidine Dose (mcg)	5.6 (7.3)	7.8 (20.1)	0.2082
Total Dexamethasone Dose (mg)	17.3 (10.3)	14.1 (11.9)	0.3731
Total Midazolam Dose (mg)	1.5 (0.8)	1.3 (1)	0.6128
Total Clonidine Dose (mcg)	1.4 (6.5)	2.8 (11.8)	0.9120
<i>Intraoperative Physiologic Variables</i>			
Heart Rate	68.6 (9.1)	69.9 (11.7)	0.7110
Invasive Arterial Blood Pressure	80.7 (5.2)	82.3 (8.4)	0.4899
Temperature	35.8 (0.9)	35.8 (0.8)	0.9921
Pulse Oximetry	98.7 (1.5)	98.9 (1.1)	0.8327
Bispectral Index	47.0 (7.1)	41.1 (6.8)	0.0810
<i>Surgery Type</i>			
1	42.9%	50.0%	
2	23.8%	33.3%	0.4798
3	33.3%	16.7%	

* p values are from *t* tests or Wilcoxon Rank sum tests for continuous variables (depending on whether the data was parametrically distributed or not), and chi-square or Fisher's exact test for categorical variables.

Table 2

Statistics for data in Fig. 2

Biomarker	Effect	Beta Coefficient	Beta SE	Effect Size (BC/BSE) *	Unadjusted <i>p</i> value	Adjusted *** value
Tau/Aβ Ratio (log transformed)	Treatment ⁺	-0.123	0.254	-0.482	0.632	1.000
	Time	0.026	0.010	2.678	5.004E-07	2.002E-06
	Interaction	0.021	0.013	1.643	0.105	0.527
Tau (log transform)	Treatment ⁺	-0.151	0.249	-0.606	0.547	1.000
	Time	0.029	0.009	3.369	3.969E-07	1.985E-06
	Interaction	0.008	0.012	0.702	0.485	1.000
Aβ	Treatment ⁺	-29.113	31.025	-0.938	0.352	1.000
	Time	-1.740	1.171	-1.486	0.076	0.152
	Interaction	0.558	1.621	0.344	0.732	1.000
p-Tau	Treatment ⁺	5.048	2.629	1.920	0.059	0.236
	Time	-0.204	0.099	-2.052	0.002	0.005
	Interaction	-0.045	0.137	-0.327	0.745	1.000
p-Tau/Aβ ratio (log transformed)	Treatment ⁺	0.281	0.147	1.908	0.061	0.295
	Time	-0.004	0.006	-0.605	0.302	0.302
	Interaction	-0.001	0.008	-0.168	0.867	1.000

* BC, beta coefficient; BSE, beta coefficient standard error.

** *p* values were adjusted with the holm method, a modification of the Bonferroni method that uses the rank order of significance to adjust *p*-values.

⁺Treatment effect Isoflurane versus Propofol.

Table 3

<i>Categorical Variables</i>	Tau Change		<i>Adjusted p value</i> **
	Median (IQR)	<i>p value</i> *	
Sex			
Male	37.5 (19, 98)	0.73	1
Female	36 (5, 112)		
Maintenance Anesthetic			
Isoflurane	56 (19, 73)	0.58	1
Propofol	20 (3, 144)		
Surgery Type			
1	36 (5, 112)		
2	70 (16, 98)	0.92	1
3	19 (5, 348)		
<i>Continuous Variables</i>	Spearman Correlation with Tau Change	<i>p-value</i>	
Case Duration (Min)	0.09	0.63	1
Patient Weight (Kg)	0.29	0.11	1
Patient BMI	0.35	0.05	0.9
Patient Age	−0.09	0.63	1
MAP mean	0.24	0.21	1
HR Mean	−0.03	0.88	1
RR Mean	−0.10	0.60	1
SPO2 Mean	0.11	0.57	1
Anesthetic Duration (Min)	0.12	0.54	1
Propofol (mg)	−0.06	0.74	1
Remifentanyl (mg)	0.05	0.78	1
Fentanyl (mcg)	0.13	0.49	1
Hydromorphone (mg)	−0.07	0.71	1
Dexmedetomidine (mcg)	0.04	0.85	1
Dexamethasone (mg)	−0.37	0.04	0.76
Midazolam (mg)	0.05	0.80	1

* Test statistics are from the Wilcoxon Rank-Sum test if the variable is binary, or the Kruskal Wallis test if the variable has more than three categories.

** *p* values were adjusted with Holm method, a modification of the Bonferroni method that adjusts for multiple comparisons using the rank order of significance.

Table 4

Genetic SNP analysis of tau change from 0 to 24 h

Gene	SNP	Minor allele	MAF	HWE**	Non-Carriers			Carriers		
					N	Mean (SD)	Median	N	Mean (SD)	Median
APOE	APOE	E4	0.09	0.93	30	88.04 (121.61)	56	7	117.75 (152.47)	61
	-219 promoter	T	0.41	0.51	14	103.36 (146.95)	26	23	77.75 (99.09)	56.5
GPI30	rs10940495	G	0.38	0.09	17	73.64 (86.16)	67	21	103.75 (148.24)	34
	rs1900173	T	0.09	1	31	89.13 (121.93)	46	7	92 (134.84)	41.5
IL6 receptor	rs2228145	C	0.37	0.08	18	69.07 (114.68)	19	20	110.33 (129.84)	73
	rs1800796	C	0.12	0.41	30	99.54 (130.36)	56.5	8	50.33 (78)	14
Tau, Exon 6	rs10445337	C	0.12	0.41	30	86.09 (117.95)	56	8	101.57 (144.64)	32
	rs2258689	C	0.21	1	23	88.41 (120.32)	56	15	91.38 (129.48)	36
TOMM40	rs157581	C	0.18	0.31	24	56.63 (81.54)	32	14	146.82 (160.17)	64

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; N, number of patients; SNP, single nucleotide polymorphism.

* Significant differences were found between the variance of the carrier and non-carrier groups. Therefore, the *p* value was based on a *t* test with unequal variance.

** Exact test was used for all SNPs except APOE, which was tested by a Chi-square test.