Human Plasma Biomarker Responses to Inhalational General Anaesthesia Without Surgery

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Abstract

Background: Postoperative neurocognitive disorders may arise in part from adverse effects of general anaesthetics on the CNS, especially in older patients or individuals otherwise vulnerable to neurotoxicity because of systemic disease or the presence of pre-existing neuropathology. Previous studies have documented cytokine and injury biomarker responses to surgical procedures that included general anaesthesia, but it is not clear to what degree anaesthetics contribute to these responses.

Methods: We performed a prospective cohort study of 59 healthy volunteers aged 40-80 yr who did not undergo surgery. Plasma markers of neurological injury and inflammation were measured immediately before and 5 h after induction of general anaesthesia with 1 minimum alveolar concentration of sevoflurane. Biomarkers included interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), C-reactive protein (CRP), and neural injury (tau, neurofilament light [NF-L], and glial fibrillary acidic protein [GFAP]).

Results: Baseline biomarkers were in the normal range, although NF-L and GFAP were elevated as a function of age. At 5 h after induction of anaesthesia, plasma tau, NF-L, and GFAP were significantly decreased relative to baseline. Plasma IL-6 was significantly increased after anaesthesia, but by a biologically insignificant degree (<1 pg ml-1); plasma TNF-α and CRP were unchanged.

Conclusions: Sevoflurane general anaesthesia without surgery, even in older adults, did not provoke an inflammatory state or neuronal injury at a concentration that is detectable by an acute elevation of measured plasma biomarkers in the early hours after exposure.

Introduction

Post-operative cognitive dysfunction (POCD) was initially conceptualized as an objective decline in cognitive function after anesthesia and surgery (1). Now termed the perioperative neurocognitive disorders (PND), these impairments were thought to be related to anesthesia medications and/or the associated physiologic derangement (2,3). Decades of research have suggested that typical physiological derangements are minor contributors, but the extent to which the anesthetic contributes remains unclear. There exists a large body of preclinical evidence that implicates the anesthetic drug itself in PND. For example, volatile drugs have been reported to enhance amyloid beta production and aggregation, tau phosphorylation and detachment from microtubules, calcium dysregulation and neuroapoptosis (4)(5)(6)(7)(8)(9). On the other hand, clinical studies have not found a consistent difference between regional and general anesthesia, or between anesthetic drugs (10)(11)(12)(13). Complicating the clinical studies, however, is the fact that almost all include a surgical procedure, which is well known to provoke a systemic inflammatory state. This inflammatory state alone could cause a cognitive syndrome of variable magnitude and duration, especially in setting of a brain rendered vulnerable by age, genetics or ongoing neuropathology or neurodegeneration.

Human biomarker studies hold promise for establishing causation as well as for risk stratification and monitoring progression of pathology. After surgery-induced tissue injury, there is an acute phase that occurs over minutes to hours, and that is marked by release of damage-associated molecular patterns (DAMPs), myriad cytokines and chemokines (14). This acute phase may

also include intense afferent vagal traffic that provokes neuroinflammation, then amplified by whatever acute phase molecules enter the brain parenchyma via a leaky blood brain barrier(15). Subsequently, vascular and neuronal injury occur, releasing a number of different (injury) biomolecules into CSF and ultimately plasma. These molecules include tau, S100β, glial fibrillary acidic protein (GFAP) and neurofilament-light (NF-L)(16). For example, several recent human studies have shown that tau and inflammatory cytokines are acutely and persistently elevated in CSF following surgery (17)(18). Further, in a series of surgical patients, plasma tau and NF-L were significantly elevated from baseline at 6 hours after the beginning of surgery (19). These biomarkers have been associated with other forms of cerebral pathology, such as traumatic brain injury, Down's syndrome, Parkinson's disease and Alzheimer's disease (AD) (20)(21)(22).

Unfortunately, the clinical studies of surgery cannot isolate a contribution of anesthesia to the biochemical outcome. We had the unparalleled opportunity in the Trajectory of Recovery in the Elderly study (TORIE) which recruited healthy volunteers, aged 40-80 years, to receive 2 hours of inhalational general anesthesia with nothing more invasive than an intravenous (IV) catheter(23). This provided us the chance to investigate whether inhalational anesthesia alone causes a similar biochemical response, such as the release of cytokines or markers of neuronal injury. Comparing this response to published data for patients receiving both anesthesia and surgery will provide insight into the major causes of biomarker release.

Methods

The parent study was approved by the institutional review board (IRB) of the Icahn School of Medicine at Mount Sinai (New York, NY; IRB@mssm.edu, 212-824-8200) and registered at ClinicalTrials.gov (NCT02275026). The primary aim of the TORIE study is to delineate the age-specific trajectory of recovery from general anesthesia in the absence of surgery and illness, full details of the parent study are described in the protocol paper (23). The primary hypothesis is that older adults exposed to general anesthesia alone will achieve complete cognitive recovery, as measured by cognitive testing, though this recovery may take longer than middle-aged adults. Secondary outcomes included the change in plasma biomarkers as reported herein. Specific inclusion criteria were adults aged 40-80, American Society of Anesthesiologists (ASA) Physical Status 1 or 2), no underlying cognitive dysfunction, as determined from baseline cognitive testing before general anesthesia. Exclusion criteria were contraindication to MRI scanning (parent study, implanted metal, claustrophobia) and pathophysiology seen on a pre-anesthesia scan that could predispose to POCD such as inflammatory conditions or cerebral microvascular disease as determined by a clinical radiologist.

Anesthesia

Anesthesia was induced in the MRI suite with propofol 2 mg/kg IV, and after induction, a laryngeal mask airway (LMA) was placed. Anesthesia was maintained with inhaled sevoflurane at an age-adjusted concentration of 1 minimum alveolar concentration (roughly 1.4 to 1.8 vol%). EEG leads were placed, and a Bispectral index of 40–60 (Covidien…) was assured after LMA placement to aid in assessment of anesthetic depth during equilibration of inhaled sevoflurane and washout of propofol. EEG leads were then removed, and the subject was returned to the MRI bore for scanning. Anesthetic depth was then monitored by end-tidal sevoflurane concentration during scanning, along with physiological measures (blood pressure, electrocardiogram, oxygen saturation, ventilation, temperature). Ventilation was maintained to achieve a target end-tidal carbon dioxide of 30–35 mm Hg. During the two-hour scanner time,

appropriate bolus administration of a vasopressor such as ephedrine (5 mg IV or 25 mg intramuscular) or phenylephrine (100 µg IV) may have been administered by the anesthesiologist to maintain mean arterial blood pressure within 20% of baseline. The participant was then removed from the MRI bore and allowed to emerge from anesthesia. The LMA was removed when the participant responded to commands. Ondansetron (4 mg, IV) was given before emergence for antiemetic prophylaxis. No narcotics, benzodiazepines, or muscle relaxants were administered.

Blood was obtained from participants on the morning of the anesthesia day, immediately prior to induction during IV placement and then again about 2 hours into the post-anesthesia care unit (PACU) stay, approximately 5 hours after the first blood draw, and the induction of anesthesia. Samples were collected in heparinized tubes, which were centrifuged. The plasma aspirated, aliquoted, rapidly frozen and stored at -80^{0}C. After accumulation of samples from 59 subjects, parallel sets of samples were sent to two labs with substantial expertise in the measurement of biofluid biomarkers.

One set of samples was shipped frozen on dry ice to the University of North Texas Health Science Center in Fort Worth, TX. Samples were inspected and accessioned into the UNTHSC Institute for Translational Research (ITR) Biomarker Core, where individual workflows for two platforms were created. The Meso Scale Discovery (MSD) platform has been used extensively to assay biomarkers associated with a range of human diseases including AD (24), (25),(26), (27) . Electrochemiluminescence (ECL) technology uses labels that emit light when electronically stimulated, which improves the sensitivity of detection of many analytes at very low concentrations. ECL measures have well-established properties of being more sensitive and requiring less volume than conventional ELISAs the gold standard for most assays(27). To further improve assay performance, assay preparation was automated using a customized Hamilton Robotics STARplus system.

Single molecule array (Simoa) technology is a fully automated immunoassay platform utilizing femtoliter sized reaction chambers to isolate and detect single proteins. Simoa technology utilizes antibody coated paramagnetic beads with biotinylated antibody detectors to simultaneously generate a detection immunocomplex that is transferred to the Simoa disc array and sealed. The presence of labeling is visualized by single molecule "on" or absence of single molecule "off" allowing for the concentration to be determined digitally rather than using traditional analog detection. Simoa technology uses enzymatically labeled antibody conjugated to magnetic beads, combined with RGP substrate to produce a fluorescence signal that is enriched in sealed micro-wells that each can hold a maximum of one bead.

Both assay platforms are reliable, showing excellent spiked recovery, dilution linearity, coefficients of variation (CV), reproducibility as well as detection limits. Internal QC protocols are implemented in addition to manufacturing protocols, including assaying consistent controls across batches and assay of pooled standards across lots. The analytic performance of each of these markers for >1300 samples across multiple cohorts and diagnoses was recently reported (normal cognition, MCI, AD) (28).

A total of 500 µL of plasma was utilized to assay the following markers (including CV and lowest level of detection [LLOD]) with CVs and LLODs calculated from the automated systems:

Meso Scale Discovery plates: C-reactive protein (CRP) (CV = 2.4; LLOD = 2.41 pg/mL), interleukin-6 (IL-6) (CV = 4.6; LLOD = 0.081 pg/mL), tumor necrosis factor α (TNFα)(CV = 3.5; LLOD = 0.077 pg/mL). Quanterix Simoa arrays: NF-L (CV =0.0374; LLOD =0.038 pg/mL), Tau (CV = 0.061; LLOD = 0.019 pg/mL).

Samples were also shipped on dry ice to the Clinical Neurochemistry Laboratory at the Institute of Neuroscience and Physiology, the Sahlgrenska Academy at University of Gothenburg, Mölndal Campus, Sweden. Similar to the above, samples were registered and assayed by the

same two platforms, with the exception that plasma NF-L was measured using an in-house Simoa method (29). Tau and GFAP were measured using commercial Simoa assays (Quanterix, Lexington, MA) as described above. IL-6, TNFα and CRP were measured using MSD assays, as described above and by the manufacturer (MesoScale Discovery, Rockville, ML). When assay measurements were determined at both labs, which was done for all markers reported herein but GFAP, the values were averaged for each participant and time point. Agreement between the two sites was high for all markers: for tau, NF-L, IL-6, TNFα, and CRP, correlation coefficients were r = 0.88, 0.94, 0.996, 0.82, and 0.95 respectively.

We experienced a freezer thaw after collecting samples from the first 29 participants that resulted in them being warmer than -80^{0}C for approximately 24-36 hours. Samples that had thawed had about 30% lower levels of TNFα but levels of other markers were not significantly affected. We included all samples, whether they had thawed or not, in all statistical analyses.

Data analysis

Statistical analyses were conducted in R version 3.5.2. Packages lme4 and lmerTest were used for linear mixed models analyses (30)(31).

Results

Participants. 788 potential participants were screened. 59 participants completed assessments through day 30 (Figure 1, CONSORT diagram). There were 34 male, 25 female, mean (SD) age 58.13 (11.6) years, mean (SD) years of education 15.3 (2.15). Pre- and post-anesthesia plasma samples were available for a total of 57 participants; post-anesthesia samples were unavailable for 2 participants.

Cytokines. To examine inflammatory processes, we measured plasma IL-6 and TNFα. These markers did not differ at baseline as a function of age: Kruskal-Wallis chi-squared (3) < 4.21, p > .24. IL-6 showed a slight, but statistically significant, increase between baseline and post-anesthesia, $F(1, \sim 54.3) = 29.3$, $p < .0005$; TNFα was unchanged, $F(1, \sim 55.0) = 0.007$, $p = .93$. Median IL-6 was 0.60 pg/mL at baseline and 1.22 pg/mL post-anesthesia; median TNFα was 2.03 pg/mL at baseline and 1.99 pg/mL post-anesthesia.

Injury biomarkers. We measured plasma tau, NF-L and GFAP as markers of neuronal injury, and CRP as a measure of vascular injury. Median (IQR) baseline plasma tau levels were 4.47 (3.51) pg/mL, median plasma NF-L levels were 15.1 (7.74) pg/mL. Baseline tau did not differ as a function of age group (decade), Kruskal-Wallis chi-squared (3) = 4.52, $p = 0.21$, but baseline NF-L increased with age, Kruskal-Wallis chi-squared (3) = 24.28, $p < .0005$. Baseline NF-L levels (median, pg/mL) were 11.9, 14.1, 16.1, 21.7 for 40-49, 50-59, 60-69, and 70-80 year-old participants, respectively. The Spearman rank correlation of baseline NF-L with age in years was 0.65, $p < .0005$.

Median (IQR) baseline levels of GFAP were 168 (106) pg/mL. As with NF-L, plasma GFAP increased with age: Kruskal-Wallis chi-squared (3) = 20.22, $p < .0005$. Baseline GFAP levels (median, pg/mL) were 148, 120, 171, 234 for 40-49, 50-59, 60-69, and 70-80 year old participants, respectively. The Spearman rank correlation of baseline GFAP with age in years was 0.51, $p < .0005$.

Median (IQR) baseline levels of CRP were 1622 (2686) ng/ml and did not differ between age groups, Kruskal-Wallis chi-squared (3) = 1.51, p = .68

Compared to baseline, plasma tau and NF-L at 5 hours after anesthesia decreased or stayed the same for most participants (Figure 3). The height of bars shows the median value at each time point, points represent data for individual participants. All 3 markers showed statistically significant decreases between baseline (T0) and ~5 hours post-induction of anesthesia (T1), linear mixed models for effect of time, Fs(1, ~54-56) > 7.45, ps < 0.009. CRP did not change between baseline and 5 hours post-anesthesia, F(1, ~55) = 0.44, p = .51.

Discussion

TORIE gave us the rare opportunity to begin to understand the biochemical response of older adults to a significant exposure to a general inhalational anesthetic alone. In these healthy volunteers, we found that plasma markers of neuronal injury, tau, NF-L, and GFAP are decreased, rather than increased, 5 hours after induction of anesthesia, as compared to baseline values. The vascular injury biomarker, CRP, was not changed. Two plasma markers that reflect the onset of inflammation, IL-6 and TNFα, were either unchanged or very slightly increased.

We chose to examine the change in NF-L and tau because they are considered sensitive markers of neuronal injury and neurodegenerative disease as demonstrated by a host of work in the Alzheimer and brain injury field (20)(21)(22). Further, a recent study has indicated that plasma NF-L and tau levels increase by 43% and 257% respectively 6 hours after the beginning of surgery in otherwise healthy older adults (32). We wanted to understand whether the injury reflected by these markers was due to anesthesia, as suggested by considerable preclinical research, or the surgical trauma itself. While our baseline values are similar, we did not see an increase in either marker. Our participants were on average younger (58 years old vs. 69 years old), however, in the 19 TORIE participants over the age of 70, we also detected no increase. Our study was limited to only two time points, while the previous study measured several values and found that plasma tau was maximally increased by 6 hours, whereas NF-L continued to slowly rise up to 48 hours. Given that both biomarkers were elevated at the 6-hour time point in the previous study, lack of an increase after anesthesia alone strongly suggests a surgical origin for inflammation rather than anesthetic. This conclusion is further bolstered by an absence of increase in ours study of two other sensitive injury biomarkers, GFAP and CRP.

The baseline values of these injury biomarkers for TORIE participants would be considered to be in the normal range, although variation existed. Most interesting was that for both NF-L and GFAP (but not tau or CRP), the levels were higher as a function of age. This has been observed before (Iverson), and may reflect a subtle, presymptomatic neurodegeneration, although not necessarily pathological. Other investigators have found that these plasma biomarkers correlate with the degree of neurodegeneration on autopsy, thus they may serve an important role in the risk stratification of patients prior to surgery, even when cognitive impairment is not detected (33).

The significant decrease in plasma neuronal injury biomarkers after 2 hours of sevoflurane was unexpected. The mechanism of this decrease can only be speculative, but might relate to a decrease in production of these biomolecules caused by a direct effect of the anesthetic, or a decrease in clearance caused by an anesthetic-induced decrease in cerebral blood flow (). Related to this might be an effect of sevoflurane on glymphatic flow and thereby clearance, although anesthetics have been thought to increase glymphatic flow, similar to natural sleep (34). It will be important to further characterize this with a more complete time course, as a consequence might be the CNS accumulation of toxic biomolecules (e.g., amyloid β).

The trigger for neuronal injury in the case of anesthesia and surgery is thought to be inflammatory. Surgery-induced tissue trauma releases a host of cellular molecules, collectively called damage-associated molecular patterns (DAMPs), that activate the innate immune system. The activation is then characterized by the release of acute phase cytokines like IL-6 and TNFα from various immune cells – both peripherally and centrally. The release after surgery is often several-fold, and highly variable amongst individuals and procedures (35). It has not been clear whether anesthesia alone causes the release of DAMPs or of cytokines in older adults. Baseline values were in the normal range, consistent with the fact that TORIE

participants were healthy. However, we were able to detect an increase in one of these cytokines (IL-6) 5 hours after the induction of sevoflurane anesthesia, but only by a very small degree (<1 pg/mL). This may reflect the minor tissue trauma of the IV insertion, and confirms that our sevoflurane exposure, even in older adults, does not activate a biologically significant inflammatory cascade.

The principal limitation of this study is the single, fairly short time interval of blood sampling, as discussed above. Further, it is possible that these markers were not sufficiently sensitive to detect a subtle stress/injury caused by the sevoflurane exposure. Also, there are important differences between our population and the general surgical population. Our volunteers were healthy and had no prior history of cognitive issues prior to surgery. Patients with a surgical disease tend to have a higher comorbidity burden than community dwelling older adults. Given that preexisting cognitive impairment is the most consistent risk factor for PND, healthy volunteers may be less vulnerable, although it is less clear that cognitive impairment is a risk factor for biomarker elevation.

In summary, 2 hours of sevoflurane general anesthesia alone in older adults produced little to no biochemical signature of either an inflammatory activation or neuronal injury when measured 5 hours after induction. Baseline values in our subjects showed an increase in NF-L and GFAP as a function of age, which may indicate utility in risk stratification. Finally, a significant decrease in neuronal injury biomarkers may reflect anesthetic-induced alterations in CNS production or clearance, which may have other implications for more delayed forms of neurotoxicity.