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ORIGINAL PAPER

Postoperative Cognitive Dysfunction is Correlated with Urine Formaldehyde in Elderly Noncardiac Surgical Patients

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Abstract Post-operative cognitive dysfunction (POCD), especially in elderly patients, has been reported in many studies. Although increasing age, duration of anesthesia, postoperative infections, and respiratory complications were regarded as the risk factors for POCD, no extracerebral diagnostic biomarkers have been identified as indicators of POCD. Ninety-five patients, ages 65–80 years, scheduled for major orthopedic or abdominal surgery were enrolled. Twenty-two patients aged between 20 and 40 years undergoing the same procedures served as controls. Subjects received neuropsychological tests one-day prior and one week post procedure. To determine the presence of POCD, the criteria were used as described in most previous studies. Morning urine samples were obtained one day before surgery and on day 1, day 2 and day 7 post operatively. Urine formaldehyde was determined with high-performance liquid chromatography. The urine formaldehyde level of all patients with and without POCD increased on the first 2 days after surgery. But the formaldehyde concentration (on day 7) in patients with POCD was significantly higher than that in patients without POCD ($p < 0.01$). In the young control group, no patient was diagnosed with POCD. Although the changes in urine formaldehyde of young patients during perioperative period were similar to those in elderly patients without POCD, the formaldehyde concentrations measured at four time points were all significantly lower than those in elderly patients ($p < 0.05$). Levels of urine formaldehyde were elevated in the perioperative period, with the highest levels at day 7 in patients with POCD. This suggests that the increase on day 7 may provide a new physiologic marker along with neuropsychological assessments to assist in the diagnosis of POCD.

Keywords Urine formaldehyde · Post-operative cognitive dysfunction (POCD) · Biomarkers

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Introduction

Post-operative cognitive dysfunction (POCD), a matter of concern for anesthesiologists and patients, is a decline in cognitive function following surgical procedures. The International Study of Postoperative Cognitive Dysfunction (ISPOCD) reported that POCD was present in 25.8 % of patients one week after surgery and in 9.9 % 3 months after surgery [1]. This cognitive dysfunction was usually exhibited by patients in terms of failure to perform simple cognitive or mental tasks that were previously easily attainable [2]. Studies on POCD have focused on the impact of increasing age or poor education, as well as perioperative factors including the types of surgery and

anesthesia, hypotension during surgery, and post-operative complications [1, 3, 4]. It also has been suggested that Alzheimer's disease (AD) is associated with POCD, and that some perioperative factors can induce POCD by activating AD neuropathogenesis [5].

It has been extensively demonstrated that exogenous formaldehyde induces memory loss in animals and human cognitive impairment as a compound with high cyto-toxicity and gene-toxicity. Exposure of rats to exogenous gaseous formaldehyde induces the accumulation of formaldehyde [6], decreases the number of hippocampal neurons ($n = 24$; $p < 0.001$), and leads to memory decline [7]. Increases in formaldehyde levels in the brain via injection of formaldehyde also lead to memory loss in rats, accompanied by decreases in the levels of neurotransmitters including acetylcholine and norepinephrine [8, 9]. Other evidence shows that formaldehyde (intraperitoneal at 10 days) induces oxidative frontal cortex and hippocampal tissue damage in rats ($n = 6$; $p < 0.05$) [10].

Previous studies showed that an increase in endogenous formaldehyde can also lead to neurodegenerative diseases [9]. Endogenous formaldehyde was present in urine, blood, and other cells including the brain. The concentration of endogenous formaldehyde tends toward homeostasis (around 0.083 mmol/L in urine) under normal physiological conditions [11]. Our previous studies showed that abnormal elevation of intrinsic formaldehyde promotes hyperphosphorylation of Tau protein and its aggregation (N2a cells at a concentration of 5×10^5 cells/ml for each) [12], accompanied by cellular dysfunction and even apoptosis [13, 14]. Significant elevation of endogenous formaldehyde in the brain occurred simultaneously with the onset of cognitive impairment in senile dementia animal models such as APP-transgenic mice ($n = 8$; $p < 0.01$), APP-PS1 transgenic mice ($n = 8$; $p < 0.01$) and SAMP-8 mice ($n = 8$; $p < 0.001$), suggesting that excess formaldehyde induced cognitive impairment [15]. In previous human studies, endogenous formaldehyde levels also were related to dementia. The concentration of urine formaldehyde of patients with medium and severe degrees of dementia is significantly greater than those of mild cognitive impairment and healthy controls ($n = 141$; $p < 0.001$) [15]. The concentration of endogenous formaldehyde in the hippocampus was increased relative to controls at autopsy ($n = 4$; $p < 0.05$) [15]. These changes showed similarity to the pathologic features of POCD. Since amine hormones are increased under the stress of surgery or trauma [16], and endogenous amines such as methylamine, can be converted to formaldehyde by the vascular enzyme semicarbazide-sensitive amino oxidase (SSAO) [17], the current study tested the hypothesis that urine formaldehyde concentration was associated with post-operative cognitive dysfunction during the postoperative period.

Methods

Subject Enrollment

Experiments were conducted with the approval of the Institution Ethics committee and after written informed consents were obtained from all the patients. Elderly patients, aged 65–80 years, scheduled for major orthopedic or abdominal surgery that was expected to last 2 h or longer, were entered sequentially into this study. In addition, there were 22 patients aged between 20 and 40 years, undergoing the same type of surgery and anesthesia served as the young control group. Exclusion criteria included an abnormal in the Mini-Mental State Examination (MMSE) before surgery, a current or past history of psychiatric or neurological disease, chronic renal disease, visual or auditory disorders, alcohol or drug dependence, illiteracy, regular use of tranquilizers or antidepressants and a history of open cardiac surgery or intracranial surgery. Patients also were excluded if they were not available to complete the neuro-cognitive test one week after surgery.

Preoperative Evaluation and Perioperative Management

Preoperative evaluation was conducted the day before surgery and included the patient's age, gender, past medical history, physical examination results, education level, recent medication history, and surgical history.

All of the patients undergoing abdominal surgery ($n = 37$) and 27 patients undergoing orthopedic surgery received general anesthesia. The general anesthesia was induced using 10 μg of sufentanil, 1.5 mg/kg of propofol, and 0.6 mg/kg of rocuronium, and was maintained with inhalation of isoflurane at 1.0–1.5 MAC (minimum alveolar concentration) during the operation. Patients were extubated in the operating room. Thirty-one patients undergoing orthopedic surgery received combined spinal epidural anesthesia. Combined spinal-epidural anesthesia (CSEA) was performed at the L2–3 or L3–4 interspace using 1 % ropivacaine, followed by insertion of an epidural catheter. The epidural catheters were removed in the PACU and all patients received patient-controlled analgesia (PCA) for post operative pain control.

Intra-operative and post-operative observations included duration of the operation and anesthesia, intra-operative and post-operative arterial blood gas analysis, blood pressure, blood loss, urine output, medicines used intra-operatively and their dosage, and post-operative delirium. Patients recorded pain intensity on a visual analog scale (VAS) with 0 representing no pain to 10 representing the most severe pain. All of the enrolled patients had similar post-surgery diets. The patients were followed daily during their hospital stay to review their medical records and check for complications.

Neuropsychological Evaluation

Neuropsychological evaluation was performed one day before and one week after surgery. The neuropsychological assessment took place in a quiet room with only the patient and an expert neuropsychologist. All assessments were completed by the same person. In total, 8 neuropsychological tests (Table 1) [18–29] were used to assess the memory and executive functions of the patients. In addition, the CAM-ICU Delirium Test, Beck Depression Inventory Test and Visual analogous scale (VAS) tests were applied to assess delirium, perioperative emotion and pain level respectively.

Diagnosis of POCD

To determine the presence of POCD, the same criteria that were used in the ISPOCD1 and ISPOCD2 studies were applied [1, 30]. We calculated the standard deviation (SD) of the differences in the results of the neuropsychological tests conducted with the same time interval from 50 age-matched healthy elderly individuals (non-hospitalized, non-surgical comparator). For the patients, the differences between baseline (pre-operative) scores and one-week post-operative scores were divided by the non-hospitalized, non-surgical control group SD to obtain a Z score for each individual test [1, 30]. Patients were diagnosed as POCD if they had Z scores greater than 1.96 in two or more tests.

Collection of Urine Samples

Urine diagnostics have several advantages including the non-invasive nature of sample collection and the presence of few interfering proteins. Since proteins interfere with formaldehyde detection by their rapid interactions, urine was analyzed instead of serum that contains high level of proteins for formaldehyde analysis.

Morning urine samples were obtained from the enrolled patients one day before surgery and on day 1, day 2 and day 7 after surgery. Each urine sample was collected before breakfast. The sample was stored in a sealed sterile container at −80 °C before analysis.

Detection of Endogenous Formaldehyde by UV-HPLC

Preparation of Urine Samples

One ml urine samples (thawed at 4 °C) were pipetted into 1.5 ml Eppendorf tubes and centrifuged (12,000 rpm, 4 °C,

Table 1 Neuropsychological tests and description

	Test	Description
1	MMSE	The Mini-Mental Stage Examination (MMSE) is the instrument most widely used in screening for cognitive problems in hospitalized patients and in outpatient settings [22]. It comprises thirty items providing information about orientation, attention, learning, calculation, delayed recall, and construction Here used as a screening test for dementia before entry to the study. The patient had to score at least 23 out of 30 possible points
2.	Hopkins verbal learning test (HVLT) with a delayed recall component	It is composed of 12 items, organized into three semantic categories, and presented over three consecutive learning trials. Twelve distractor items (6 semantically related and 6 semantically unrelated) are interspersed with the 12 test items during subsequent immediate yes/no recognition testing [23]
4	Revised brief visuospatial memory test (BVMT-R) with a delayed recall component	In this test, patients are presented with a plate containing six geometric visual designs in a 2 × 3 matrix. The stimulus is presented for 10 s and patients are instructed to reproduce as many designs as possible after the stimulus is removed from view. After 25 min delay, the task is repeated [24]
4.	Trail making test (TMT)	It consists of two parts: On TMT Part A subjects have to connect numbers from 1 to 25, which are randomly spread over a sheet of paper, in ascending numerical order. On part B, participants are asked to connect randomly spread numbers (from 1 to 13) and letters (from A to L) in alternating numeric and alphabetical order (1-A-2-B-3-C-…-13-L) [25]
5.	Digit span test (DST)	It is a common measure of short-term memory, i.e. the number of digits a person can absorb and recall in correct serial order after hearing them or seeing them [26]
6.	Benton judgment of line orientation	The test consists of line segments of varying spatial orientation which must be matched with a set of longer lines on a response card [27]
7.	Digit symbol-coding test	Patients are shown a list of digits and symbols and immediately asked to write down the symbols that correspond to a list of digits [28]
8.	D-KEFS verbal fluency test	This test is composed of three conditions: letter fluency, category fluency, and category switching [29]

10 min). A 0.4 ml aliquot of the supernatant was taken to mix with 2,4-dinitrophenylhydrazine (DNPH, final concentration 0.1 g/L, in acetonitrile) and 0.1 ml of trichloroacetic acid. Samples were vortexed vigorously for 30 s before centrifugation (12,000 rpm, 4 °C, 10 min). Supernatants were added to 2-ml glass vials and heated in a 60 °C water bath for 30 min, followed by ultra-filtration (0.22 nm) at room temperature before being subjected to high-performance liquid chromatography (HPLC). The HPLC system (LC-20A, Shimadzu, Japan) was equipped with an ultraviolet detector. Urine from healthy subjects was used as a control.

Analysis of Formaldehyde in Urine by HPLC

Following the method described by Shara et al. [31], formaldehyde in the urine samples was allowed to react with DNPH and analyzed for formaldehyde concentration using HPLC [32]. The formaldehyde-DNPH derivative was eluted from the HPLC column at a retention time of 7 min. Methanol, formic acid, acetaldehyde, and other aldehyde compounds did not interfere with the analysis of formaldehyde. Laboratory analyses and clinical investigations were carried out in a double-blind manner.

Calculation of Urine Formaldehyde Concentration

Urine samples were highly diluted because of postoperative fluid infusions and urine-creatinine concentrations were used to adjust for the value in the diluted urine samples [33]. The formaldehyde-to-creatinine ratio (F/C ratio) was used to correct the formaldehyde results in the diluted urine. Adjustment by creatinine gives a more objective estimate of urine formaldehyde concentration [34, 35]. For convenience, we refer to this as the "formaldehyde concentration (FA)" instead of the adjusted formaldehyde concentration unless otherwise stated. Assay of urine creatinine was carried out using a UniCel® DxC 800 Synchron® Clinical System (Beckman, USA).

Statistical Analysis

Quantitative and categorical data were compared between patients with and without POCD using analysis of variance (ANOVA), Fisher's exact test, and nonparametric tests. Changes in urine formaldehyde concentration were analyzed by Independent-Sample *t* tests and multivariate tests of repetitive measure ANOVA. Statistical analysis was conducted with SPSS for Windows (version 13.0, International Business Machines Corporation, USA).

Results

Diagnosis for POCD with Neuropsychological Evaluation

A total of 120 patients were approached for consent, and 117 agreed. In the elderly group, 30 cases (31.6 %) were diagnosed with POCD one week after their surgery. No significant difference was observed between patients with and without POCD in age, gender, education level, ASA classification, past medical history, type of surgery and anesthesia ($p > 0.05$), as shown in Table 2. While in the young control group, no patient was diagnosed with POCD.

Neuropsychological assessment of POCD patients revealed that 93.3 % had a decreased performance in short-term memory tests (Hopkins Verbal Learning Test [HVLT] or Brief Visuospatial Memory Test [BVMT-R]), 73.3 % had decreased in delayed recall tests (HVLT Delayed Recall or BVMT Delayed Recall), and 70.0 % had declined in the Trail Making Test.

There was no intra-operative awareness experienced during this study. Six patients experienced hypotension during surgery, four of whom had a brief episode of hypotension, which was quickly corrected by administration of ephedrine. Three patients experienced mild depression before surgery and one day after surgery. The occurrence of POCD had no significant correlation with surgical duration, blood loss, the presence of hypotension, hypoxia, hypercapnia, delirium, post-operative complications, depression status, or pain score ($p > 0.05$, Table 2).

Changes in Urine Formaldehyde of Patients with and without POCD After Surgery

As shown in Fig. 1, in the elderly group, urine formaldehyde concentrations of patients with and without POCD both increased markedly after surgery compared with baseline levels detected 1 day before surgery. However, urine formaldehyde concentrations of patients with or without POCD were not significantly different on day 1 and day 2, but POCD patients had significantly ($p < 0.01$, 30/65, Fig. 1A) higher levels on day 7.

Considering the individual variation in urine formaldehyde among different patients, we defined a ratio $[FA]_A/[FA]_B$, where $[FA]_A$ and $[FA]_B$ represent FA concentrations after and before surgery respectively (Fig. 1b). The increased $[FA]_A/[FA]_B$ ratio of patients with POCD was more significant ($n = 30$, $p < 0.001$) than that of the normal group ($n = 65$) on day 7 after the surgery. This indicates that the $[FA]_A/[FA]_B$ ratio on day 7 was related to cognitive decline.

Twenty-five of the elderly patients had cancer. Urine formaldehyde was remarkably elevated in these cancer

Table 2 Medical and demographic characteristics of patients with and without post-operative cognitive dysfunction (POCD)

	Patients with POCD (n = 30)	Patients without POCD (n = 65)	*P* value
Demographics			
Age	70.8 ± 4.1	71.5 ± 4.0	0.684
Male	40.0 % (n = 12)	33.8 % (n = 22)	0.561
Years of education	8.9 ± 3.4	9.8 ± 4.0	0.132
Medical history			
Cancer	20.0 % (n = 6)	29.2 % (n = 19)	0.342
Hypertension	50.0 % (n = 15)	50.8 % (n = 33)	0.944
Diabetes	26.7 % (n = 8)	20.0 % (n = 13)	0.467
Coronary disease	16.7 % (n = 5)	12.3 % (n = 8)	0.541
Past surgery	33.3 % (n = 10)	41.5 % (n = 27)	0.446
Perioperative factors			
ASA			
I	20.0 % (n = 6)	18.5 % (n = 12)	0.872
II	43.3 % (n = 13)	50.8 % (n = 33)	
III	33.3 % (n = 10)	30.8 % (n = 20)	
Surgery			
Orthopedic surgery	66.7 % (n = 20)	58.5 % (n = 38)	0.446
Abdominal surgery	33.3 % (n = 10)	41.5 % (n = 27)	
Anesthesia			
GA	73.3 % (n = 22)	70.8 % (n = 46)	0.797
CSEA	26.7 % (n = 8)	29.2 % (n = 19)	
Benzodiazepines before surgery	76.7 % (n = 23)	73.8 % (n = 48)	0.769
Operation duration			
2–3 h	60.0 % (n = 18)	64.6 % (n = 42)	0.896
3–4 h	33.3 % (n = 10)	21.5 % (n = 14)	
> 4 h	6.7 % (n = 2)	13.8 % (n = 9)	
Blood loss			
< 500 ml	83.3 % (n = 25)	78.5 % (n = 51)	0.637
500–1000 ml	10.0 % (n = 3)	16.9 % (n = 11)	
> 1000 ml	6.7 % (n = 2)	4.6 % (n = 3)	
Hypotension			
No hypotension	96.7 %(n = 29)	92.3 %(n = 60)	0.442
Transient*	0 % (n = 0)	6.2 % (n = 4)	
Continuous**	3.3 % (n = 1)	1.5 % (n = 1)	
Hypoxia	0 % (n = 0)	0 % (n = 0)	–
Hypercapnia	3.3 % (n = 1)	0 % (n = 0)	0.316
Delirium	0 % (n = 0)	1.5 % (n = 1)	1.000
Complications	20.0 % (n = 6)	26.2 % (n = 17)	0.515
Pain			
VAS (1st day after surgery)	6.4 ± 1.4	6.0 ± 1.4	0.694
VAS (7th day after surgery)	2.4 ± 1.0	2.2 ± 0.7	0.174
Depression			
Before surgery	3.3 % (n = 1)	3.1 % (n = 2)	1.000
After surgery	0 % (n = 0)	1.5 % (n = 1)	1.000

GA general anesthesia, *CSEA* combined spinal-epidural anesthesia, *VAS* visual analogous scale

* Four patients had a brief episode of hypotension during surgery which was quickly corrected by administration of ephedrine

** Two patients had continuous hypotension during surgery

patients, consistent with previous reports [36]. When cancer patients were excluded, the difference of both FA concentration and the $[FA]_A/[FA]_B$ ratio between subjects with and without POCD became more significant (n = 24, $p < 0.001$) (Fig. 1c, d). This suggests that, with the exception of cancer patients, the $[FA]_A/[FA]_B$ ratio can be

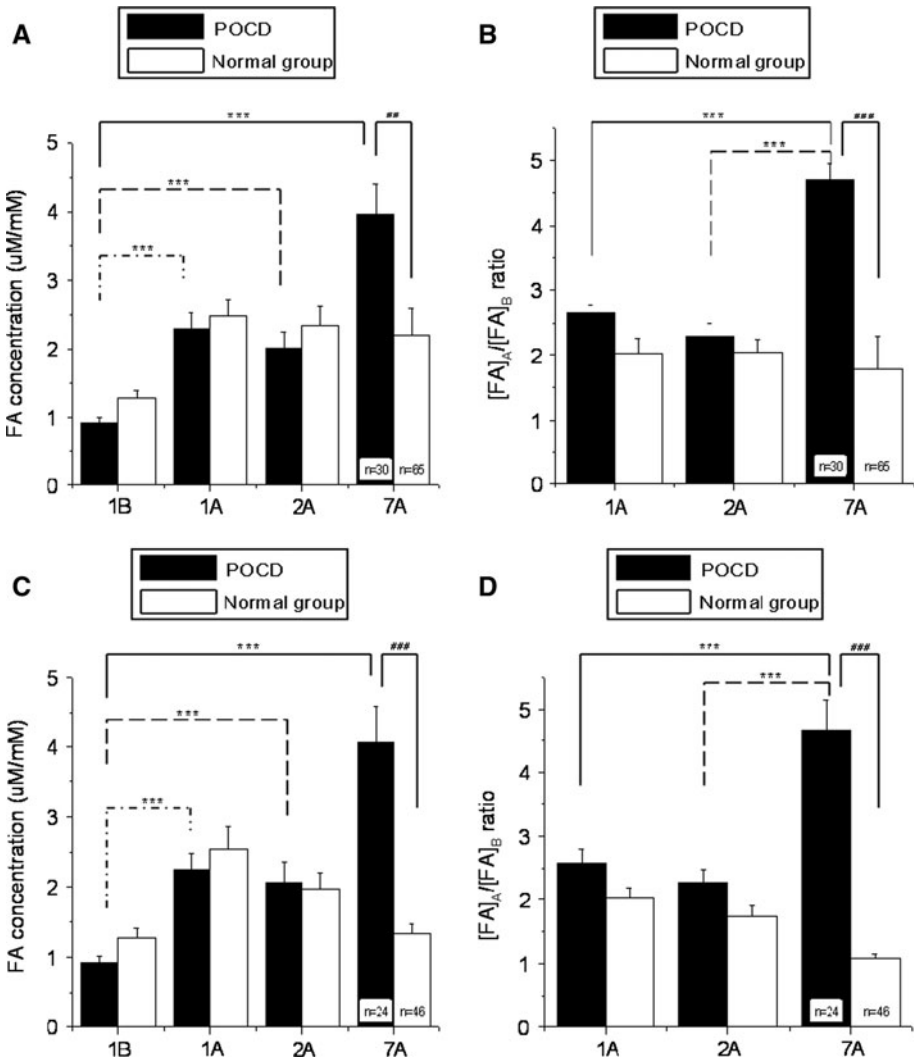

Fig. 1 Urine formaldehyde concentrations in elderly enrolled patients. Midstream morning urine (10 ml) was collected from patients before and after surgery (day 1, day 2, and day 7). The urine formaldehyde level was analyzed by UV-HPLC. The urine formaldehyde-to-creatinine ratio (F/C ratio) was used to correct false positive or false negative formaldehyde results caused by variation in urine concentration. The changes (Mean ± SEM) were shown in FA concentration of all enrolled elderly patients with and without POCD (**a**), as well as those excluding patients with cancer (**c**). The $[FA]_A/[FA]_B$ ratio is shown in **b** and **d.** Comparison of urine FA concentration of patients: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ by multi-variate tests of repetitive measure ANOVA and Independent-Sample *t* tests. *1B* 1 day before surgery; *1A*, *2A* and *7A* 1 day, 2 days and 7 days after surgery, respectively. *FA* formaldehyde

used as a criterion to support neuropsychological tests in the diagnosis of POCD.

Correlation of Formaldehyde Concentrations with Types of Surgery and Anesthesia

All enrolled elderly patients were divided into different groups according to the types of surgery and anesthesia. Perioperatively, no significant differences in $[FA]_A/[FA]_B$ ratios were observed between abdominal and orthopedic surgery. Furthermore, the $[FA]_A/[FA]_B$ ratios between the general anesthesia group (GA; n = 31) and the combined spinal epidural anesthesia group (CSEA; n = 27) do not differ significantly on day 1 and day 2. Although the ratio $[FA]_A/[FA]_B$ of GA seemed to be higher than that of CSEA, it was not significantly different ($p > 0.05$). This suggests that the types of surgery and anesthesia do not significantly contribute to the increase in the endogenous formaldehyde and the $[FA]_A/[FA]_B$ ratio of the elderly patients with cognitive impairments after their surgery.

Changes in Urine Formaldehyde of Young and Elderly Patients without POCD and Cancer After Surgery

As described in the introduction, urine formaldehyde has been shown to be correlated with age. Unpublished preliminary data from our epidemiological study showed that the formaldehyde urine concentration (n = 32, 0.032 ± 4 μM) of young people (22–29 years old graduate students) was significantly lower ($p < 0.05$) than that (n = 35, 0.041 ± 5 μM) of an elderly group (65–75 years old retired in Beijing). In the present surgical study, comparing changes of formaldehyde in young and elderly patients without POCD and cancer, the urine formaldehyde concentrations of the young patients during the perioperative period were significantly ($p < 0.001$) lower than those of the elderly patients (Fig. 2a). Furthermore, the urine formaldehyde levels of the young group increased prominently on day 1 and day 2 after surgery, but decreased to the baseline level on day 7 after surgery, which was consistent with the elderly patients without POCD and cancer.

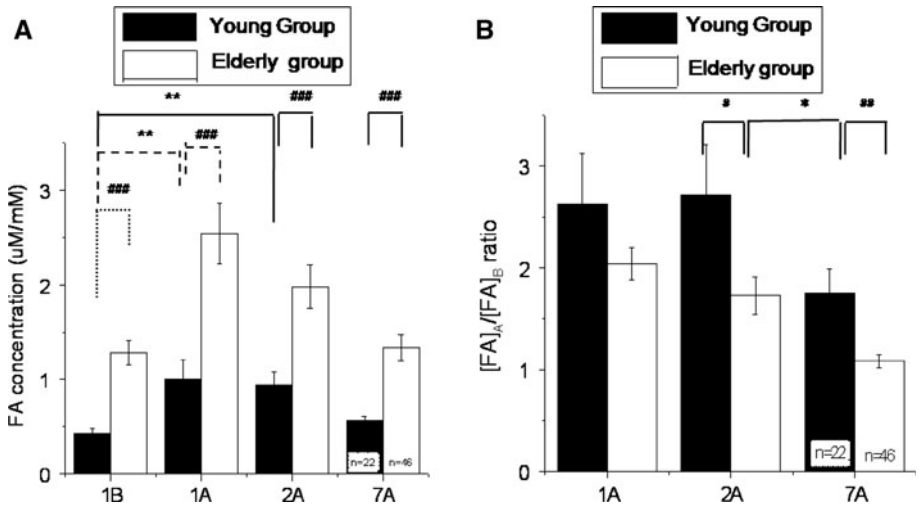

Fig. 2 The effect of age on urine formaldehyde concentrations in enrolled patients. The changes in FA concentrations and $[FA]_A/[FA]_B$ ratio of young group and elderly group without POCD and cancer were shown in (**a**) and (**b**)

The $[FA]_A/[FA]_B$ ratios decreased significantly ($p < 0.05$) on day 7 after surgery in both young and elderly groups without POCD (Fig. 2b). The $[FA]_A/[FA]_B$ ratios of the young patients were higher than the elderly patients because of the lower urine formaldehyde baseline of young people.

Discussion

Formaldehyde is produced constitutively in human cells. Organelles within living cells, including the nucleus, cytoplasm, mitochondria, endoplasmic reticulum, and membranes continuously synthesize and release formaldehyde [37]. The level of human endogenous formaldehyde is maintained at a low concentration (around 0.083 mmol/L in urine) under physiologic conditions, but the concentration increases with aging (over 65 years old) [11]. Endogenous methylamine can be converted to formaldehyde by SSAO [17]. Several enzymes in the liver including aldehyde dehydrogenase class 2 (ALDH2) and alcohol dehydrogenase (ADH1) can catalyze the reaction that oxidizes formaldehyde to formic acid [38]. Catalysis of the conversion of formaldehyde to formate via glutathione-dependent formaldehyde dehydrogenase (FDH; also known as class III alcohol dehydrogenase, ADH3), takes place in all tissues of the human body as part of the regulation of endogenous formaldehyde [39]. By this pathway, endogenous or exogenous formaldehyde is eliminated from the body as metabolites, primarily as formate or CO_2 [40].

Several mechanisms may lead to the elevation of formaldehyde levels in elderly patients by inducing an imbalance between the production and degradation of endogenous formaldehyde. (1) Metabolic efficiency of the elderly patients begins to slow down [41], especially after the stress of surgery [42, 43]. (2) Oxidative stress was identified in trauma and surgical patients [44]. Reactive carbonyl compounds (RCCs) including formaldehyde [45], acetaldehyde [46], acrolein and malonaldehyde [47] are products of oxidative stress. (3) Mitochondria are deeply activated in the process of oxidative stress. Cytochrome P-450 mediated oxidation is involved in the formation of endogenous formaldehyde [48]. Excess endogenous formaldehyde could induce a metabolic response and abnormal modifications of cellular proteins such as hydroxymethylation and hyperphosphorylation, protein misfolding, nuclear translocation, and even cell death [11].

Formaldehyde is able to penetrate the blood–brain barrier [49], and is eliminated from the body through urine and respiration in addition to enzymatic catalytic pathways [50]. As an active organic molecule, formaldehyde reacts rapidly with protein α-/ε-amino groups. Since there are fewer proteins in urine than in serum to react with formaldehyde, measurement of urine formaldehyde by reacting with DNPH is much less affected than that of serum formaldehyde. Urine is also much more convenient to sample and store than blood, CSF, or brain tissue. Thus, this study may provide a non-invasive way to diagnose POCD by using urine formaldehyde as a biomarker.

In this study, it was observed that levels of urine formaldehyde were remarkably elevated in all patients after surgery. The formaldehyde concentration (on day 7) in patients with POCD was significantly higher than that in patients without POCD. Changes in urine formaldehyde were shown to correlate with the occurrence of cognitive impairment. The $[FA]_A/[FA]_B$ ratio decreased the variations between different individuals, and the ratios of patients with POCD were higher than those without POCD. This supports the conclusion that excess endogenous formaldehyde is related to the pathogenic processes of POCD.

In the young patients, no one was diagnosed with POCD. The changes in perioperative urine formaldehyde

of young patients were similar to those of the elderly patients without POCD, although the formaldehyde concentrations measured at four points of time were all significantly lower than those of the elderly group. According to epidemiologic data, the endogenous level of formaldehyde in young people is significantly lower than that of elderly people. As described previously, amine hormones are increased under the stress of surgery or trauma [16] and endogenous amines such as methylamine can be converted to formaldehyde by SSAO. Other studies showed that overexpression of SSAO [51] and deficiency of ALDH [38, 52] are associated with an increased risk of dementia. This suggests that the potential to scavenge increased endogenous formaldehyde plays an important role in the prevention of POCD. The elderly patients were much more susceptible to POCD than young patients due to their higher levels of endogenous formaldehyde. In addition, metabolic efficiency of the elderly patients begins to slow down [41], especially after the stress of surgery [42, 43]. Thus, the elderly patients with dysfunction in the clearance of endogenous formaldehyde after surgery would be at a greater risk of POCD.

There was no significant difference in the $[FA]_A/[FA]_B$ ratios of the enrolled patients with orthopedic surgery or abdominal surgery. In addition, the $[FA]_A/[FA]_B$ ratio did not vary significantly between the GA and CSEA groups, with both POCD and non-POCD patients. Different types of anesthesia may not be significantly involved in the metabolic changes of the endogenous formaldehyde. This suggests that changes in the $[FA]_A/[FA]_B$ ratios under the stress of surgery are more closely related to POCD than to the types of surgery and anesthesia.

Proliferating cancer cells secrete excess formaldehyde [36]. It should be noted that the perioperative urine formaldehyde level of patients with cancer was remarkably high. As shown in Fig. 1c, the $[FA]_A/[FA]_B$ ratio in 7 cancer patients among 65 for normal elderly patients was higher than the average ratio for POCD patients (4.71) on day 7 after surgery. Excluding subjects with cancer, the $[FA]_A/[FA]_B$ ratio for patients with POCD was 4.66 ± 0.49 ($n = 24$). None of the subjects without POCD ($n = 46$) had ratios greater than 4.66. This shows that the $[FA]_A/[FA]_B$ ratio could be a marker to be used alongside neuropsychological tests in the diagnosis of POCD in non-cancer surgical patients.

In this study, changes in urine formaldehyde concentrations were measured on day 1, day 2 and day 7 after surgery. We think that the changes in the concentration of urine formaldehyde at one week should reflect POCD. This viewpoint is based on these observations. (1) The urine formaldehyde concentration of the young patient control group increased and then decreased after surgery, and the value became similar to that before surgery (Fig. 2a). (2) Furthermore, the concentration of urine formaldehyde of the elderly group without POCD as an age-matched control also decreased to the level before surgery. (3) Urine formaldehyde concentrations and the $[FA]_A/[FA]_B$ ratios of patients with or without POCD were not significantly different on day 1 and day 2, but were significantly different on day 7 (Fig. 1a, b). (4) The incidence of POCD was 31.6 % for the elderly group one week after surgery, compared to that (25.8 %) reported by Moller and colleagues [1]. These data indicate that the urine formaldehyde concentration on day 7 may serve as a biomarker of POCD.

Risk factors for early POCD include increasing age, duration of anesthesia, poor education, second operations, and post-operative complications [1]. The data presented here indicate another potential risk factor for POCD may be endogenous formaldehyde, which is capable of exerting effects on neural function leading to learning decline and memory loss.

These findings should be interpreted within the constraints of the study's potential limitations. Since early POCD is usually diagnosed on the seventh day after surgery, the patients were not followed up and tested at 3 months. In future studies, patients should be followed-up longitudinally.

This is the first study showing the correlation between endogenous formaldehyde and POCD. Based on the current results, it is concluded that in the first week after major noncardiac surgery, the patients with POCD were detected a higher urine $[FA]_A/[FA]_B$. The results thus suggest that the ratio $[FA]_A/[FA]_B$ in urine on day 7 may serve as a potential marker for POCD. This work also raises the possibility that urine formaldehyde could be used as a marker for the assessment of therapeutics in POCD.

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