The association of plasma gamma-aminobutyric acid concentration with postoperative delirium in critically ill patients

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ABSTRACT

Objective: Delirium is a common complication in postoperative, critically ill patients. The mechanism of postoperative delirium is not well understood but many studies have shown significant associations between benzodiazepine use, alcohol withdrawal and cirrhosis, and an increased risk of delirium. We aimed to investigate a possible link with alterations of gamma-aminobutyric acid (GABA) activity.

Design, setting and participants: A prospective observational investigation of 40 patients > 20 years old who had undergone elective surgery with general anaesthesia and were expected to need postoperative intensive care for more than 48 hours. We assessed postoperative delirium using the confusion assessment method in the intensive care unit at 1 hour after the operation and on postoperative Day (POD) 1 and POD 2. We collected blood samples for measurement of plasma GABA concentrations before the operation and on POD 1 and 2.

Main outcome measures: Postoperative delirium and perioperative plasma GABA concentrations in patients with and without delirium.

Results: Postoperative delirium occurred in 13 of the patients. Patients with delirium had significantly higher Acute Physiology and Chronic Health Evaluation II scores than patients without delirium. The mean plasma GABA concentration on POD 2 was significantly lower in patients with delirium than in those without delirium. After adjustment of relevant variables, plasma GABA concentration on POD 2 was independently associated with postoperative delirium.

Conclusions: Plasma GABA level on POD 2 has a significant independent association with postoperative delirium.

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Delirium often occurs postoperatively in critically ill patients[1,2] and appears to be correlated with increased rates of morbidity and long-term cognitive impairment.[3] However, its aetiology and pathophysiology are still unclear.[4]

Gamma-aminobutyric acid (GABA) is an amino acid synthesised from glutamate.[5] GABA is the most important inhibitory transmitter and is predominantly distributed in the cortex, basal ganglia, hippocampus, hypothalamus, amygdala, cerebellum, medulla and spinal cord.[6] Alteration of GABA activity was reported in patients being treated with benzodiazepines, patients undergoing alcohol withdrawal and patients with cirrhosis,[7-9] and many studies have shown that such patients have a significantly higher risk of delirium.[10-12] These facts suggest a possible link between alterations of GABA activity and delirium. GABA concentration in cerebrospinal fluid is thought to reflect changes in central GABA neurotransmission,[13,14] and plasma GABA concentration may be a useful marker of brain activity related to GABA.[15] However, there has been no study in which the association of perioperative plasma GABA concentrations with postoperative delirium was assessed.

Our study was a prospective, observational assessment of the association of perioperative plasma GABA concentration with delirium in postoperative patients, and was conducted simultaneously with an already published study to assess the association between melatonin and delirium.[16] Our null hypothesis was that there is no significant association between preoperative plasma GABA concentration and delirium that develops within 48 hours after the operation.

Materials and methods

Our study was conducted simultaneously with another study.[16] This study included the same patients, therefore, most of the methods are the same as those in the other study.

Study design

This was a prospective, observational investigation conducted in a tertiary teaching hospital with 22 beds in the intensive care unit. It was approved by the human research ethics committee of Okayama University Hospital. Written, informed consent was obtained from all patients. The trial was registered with clinicaltrials.gov (NCT01570881).

Patients

Included were patients aged over 20 years who had undergone elective surgery with general anaesthesia from 1 April 2010 to 31 January 2011 and who were expected to

Table 1. Schedule for testing delirium and GABA

	Day of operation		Postop. Day 1		Postop. Day 2	
Test	8 am preop.	1 hour postop.	8 am	5 pm	8 am	5 pm
Delirium	–	•	•	•	•	•
GABA	•	–	•	–	•	–

Postop. = postoperative. Preop. = preoperative. GABA = gamma-aminobutyric acid.

require postoperative intensive care for more than 48 hours. Exclusion criteria included a requirement for emergency surgery, cardiopulmonary bypass surgery or brain surgery; a history of psychosis, dementia or drug or alcohol misuse; or a vision or hearing impairment.

Patients undergoing postoperative mechanical ventilation (MV) were administered propofol 1–3 mg/kg/hour. Within the study period (the first 48 hours after operation), no benzodiazepines were used. The decision to wean a patient off MV was made by the attending doctors. The doctors were blinded to the results of the delirium tests, which used the confusion assessment method in the ICU (CAM-ICU), and were also blinded to the plasma GABA concentrations. Protocols for standard anaesthesia and decisions about discharge from the ICU were the same as those in the earlier study[16] (see the Appendix).

Diagnosis of postoperative delirium and measurement of plasma GABA concentration

In the study period, one doctor (S Y), who was trained in the use of the CAM-ICU test, performed five assessments of delirium using the CAM-ICU test at 1 hour after the operation and at 8 am and 5 pm on postoperative Day (POD) 1 and POD 2 (Table 1). We defined patients with delirium as those with positive CAM-ICU scores in at least one of the five assessment areas.

We collected blood samples for measurements of plasma GABA concentrations at 8 am before the patient's operation and on POD 1 and POD 2 (Table 1). Plasma was separated by centrifugation and stored at –30°C in a polypropylene tube until the time of assay. Plasma GABA concentrations were measured by o-phthalaldehyde fluorescence postcolumn derivation, using a high-performance liquid chromatography method (FP-820 fluorescence spectrofluorometers and PU-880 HPLC system, JASCO).

Patient demographics

We obtained data for patient demographics including age, sex, Acute Physiology and Chronic Health Evaluation (APACHE) II score,[17] Charlson comorbidity index,[18] preoperative use of benzodiazepines, surgical category, surgical duration, estimated intraoperative blood loss, requirement of postoperative MV and length of ICU stay.

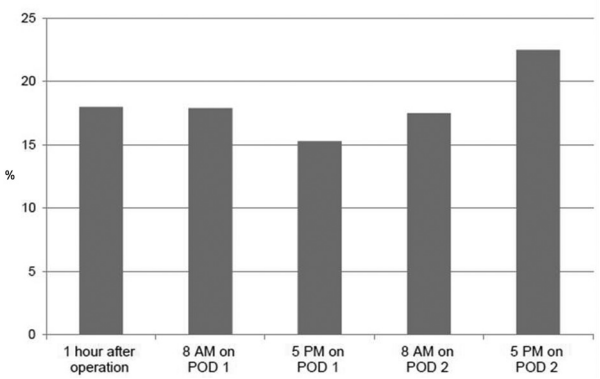
Statistical analysis

The primary outcome was postoperative delirium. Demographic variables and plasma GABA concentrations were summarised using proportions or medians with interquartile ranges (IQRs) as appropriate, and compared using the χ^2 test and Wilcoxon rank-sum test in patients with and patients without delirium. To calculate the sample size for the current trial, we considered a difference of 25 pmol/mL in plasma GABA concentration to be meaningful. Assuming a standard deviation of 25 pmol/mL, an incidence of delirium of 30%, a power of 0.80, and an α level of 0.05, 40 participants were required.

To determine the independent contribution of plasma GABA concentration to the prediction of postoperative delirium, we constructed multivariate models using potential predictors of delirium (criteria for inclusion, $P = 0.05$). Results from the multivariate models are shown using odds ratios (ORs) with 95% confidence intervals. We determined model calibration using the Hosmer–Lemeshow test for goodness of fit. We tested for multicollinearity using the variance inflation factor. All variance inflation factors were less than 5, indicating absence of severe multicollinearity.

$P < 0.05$ was considered statistically significant. All statistical analyses were performed using commercially available statistical software (SPSS version 19.0, SPSS Inc.). Data were reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[19]

Figure 1. Incidence of postoperative delirium at each assessment, using CAM-ICU at each assessment performed*



CAM-ICU = confusion assessment method in the intensive care unit. POD = postoperative day. * There was no significant difference in the incidence of delirium among five assessments ($P > 0.99$). We defined patients with delirium as those with positive CAM-ICU in at least one of the five assessments. Postoperative delirium occurred at least once in 13 of the patients (33%).

Table 2. Comparison of demographic data of patients with delirium ($n = 13$) and without delirium ($n = 27$)

Characteristic	With delirium	No delirium	*P*
Age, years*	73 (63–77)	64 (57–73)	0.083
Male†	11 (85%)	22 (81%)	0.81
APACHE II score*	18 (16–21)	13 (12–16)	0.001
Charlson comorbidity index*	2 (1–2)	2 (1–3)	0.58
Preop. benzodiazepines†	3 (23%)	10 (37%)	0.51
Surgical category†			
Abdominal	4 (31%)	12 (44%)	0.41
Thoracic	4 (31%)	8 (30%)	0.94
Otolaryngologic	5 (38%)	6 (22%)	0.29
Spinal	0 (0%)	1 (4%)	0.49
Operation time, minutes*	530 (412–643)	401 (303–537)	0.07
Est. intraop. blood loss, mL*	430 (300–575)	420 (230–825)	1
Postop. MV†	9 (69%)	10 (37%)	0.059
Postop. MV time, hours*	38.8 (17.7–62.4)	16.3 (13.7–95.2)	0.35
Length of ICU stay, days*	3 (3–7)	3 (2–6)	0.38

IQR = interquartile range. APACHE = Acute Physiology and Chronic Health Evaluation. Preop. = preoperative. Est. = estimated. Intraop. = intraoperative. Postop. = postoperative. MV = mechanical ventilation. ICU = intensive care unit. * Median and interquartile range.† Percentage.

Table 3. Multivariate logistic regression analysis for plasma GABA concentration on postoperative Day 2

Variable	Odds ratio (95% CI)	*P*
APACHE II score	1.41 (1.04–1.9)	0.027
GABA on Day 2	0.95 (0.91–1)	0.043

GABA = gamma-aminobutyric acid. APACHE = Acute Physiology and Chronic Health Evaluation.

Results

We included the same patients as those in an earlier study.[16] We obtained written informed consent for participation from 62 patients. Twenty-two patients were excluded due to cancellation of surgery or a short duration of ICU stay (< 48 hours). Thus, 40 patients were included and all completed the study to follow-up.

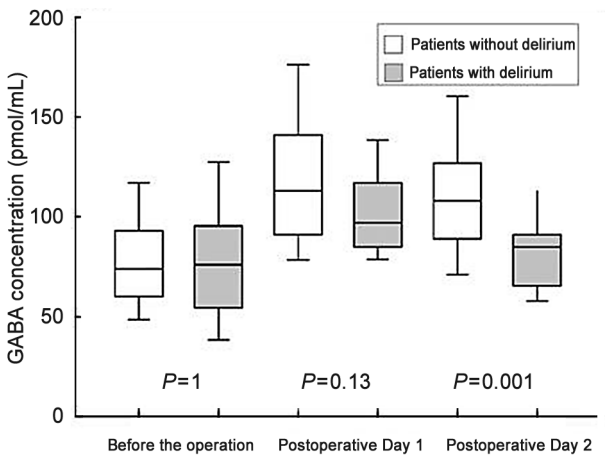
Figure 1 shows the incidence of delirium at each assessment. There was no significant difference in the incidence of delirium among five assessments ($P > 0.99$). Postoperative delirium occurred at least once in 13 (33%) of the patients.

Table 2 shows the comparison of patient demographic data between patients with and without postoperative delirium. The APACHE II score was significantly higher in patients with delirium ($P > 0.001$). Other demographic data including age, sex, Charlson comorbidity index,[18] preoperative use of benzodiazepines, operation category, operative duration, estimated intraoperative blood loss and requirement of postoperative mechanical ventilation were not significantly different between patients with and without delirium, as we reported previously.[16]

Figure 2 shows a comparison of plasma GABA concentrations in patients with and without delirium. The median plasma GABA concentrations in patients with delirium (before their operation, 76 pmol/mL; POD 1, 97 pmol/mL) ($P = 1$) was not significantly different from those in patients without delirium (before their operation, 74 pmol/mL; POD 1, 113 pmol/mL) ($P = 0.13$). The median GABA concentration on POD 2 was significantly lower in patients with delirium than in patients without delirium (85 pmol/mL v 108 pmol/mL, $P = 0.001$).

Since the severity of illness may contribute to the association of plasma GABA concentration on POD 2 with delirium, we performed a multivariate analysis to assess the independent associations with the risk of delirium. Even after adjustment for APACHE II scores, a lower plasma GABA concentration on POD 2 was independently associated with an increased risk of postoperative delirium (adjusted OR, 0.95, $P = 0.043$) (Table 3). This model was a good fit for data (Hosmer–Lemeshow test, $P = 0.64$).

Figure 2. Plasma gamma-aminobutyric acid (GABA) concentrations in patients with and without delirium



Boxes represent interquartile ranges. Horizontal lines in middle of boxes are medians. Vertical H-Bars are upper and lower adjacent values. GABA concentration on postoperative Day 2 was significantly lower in patients with delirium than in patients without delirium (85 pmol/mL v 108 pmol/mL; $P = 0.001$).

Since patients with delirium tended to more frequently need postoperative MV, we also performed an analysis to assess their independent associations with the risk of delirium. Even in this sensitive analysis, we found a significant independent association with delirium (adjusted OR, 0.95, $P = 0.044$). This model was also a good fit for data (Hosmer–Lemeshow test, $P = 0.64$)

Discussion

Main findings

This study was a prospective observational study to assess the association of perioperative alteration of plasma GABA concentration with delirium in postoperative patients who needed intensive care for more than 48 hours. Although preoperative GABA concentrations were not significantly different, GABA concentrations on POD 2 were significantly lower in patients with delirium than in patients without delirium. Even after adjustment for severity of illness, a lower plasma GABA concentration on POD 2 was independently associated with an increased risk of postoperative delirium.

Limitations

Our study has several limitations. First, it was an observational study and thus showed an association but not a causal link. Second, this was a small, single-centre study with a chance of a type I error and weak generalisability. Thus, our findings should be confirmed or refuted by future studies. Third, we measured GABA concentrations at three time points, which may introduce error due to multiple comparisons. If we define statistical significance as $P < 0.017$ (0.05/3), the GABA concentration still significantly differed between patients with and without delirium. Fourth, our study was conducted for 48 hours, which might be too short to observe the perioperative alteration of plasma GABA concentration. Thus, observation for a longer period should be conducted in a future study. However, it should be noted that there has been no study in which alterations in plasma GABA concentrations in patients who needed postoperative intensive care were investigated. In this regard, this study may provide a clue for research on perioperative plasma GABA concentrations. Finally, we included patients on and not on postoperative MV, which might have affected the accuracy of diagnosis of delirium and confounded our findings. However, we reported an independent association of plasma GABA concentration on POD 2 with delirium even after adjusting for the effect of MV.

Comparison with earlier studies

To our knowledge, our study is the first to assess the association of perioperative plasma GABA concentration with the risk of delirium. Thus, our findings cannot be compared with those of any other studies in an acute-care setting.

Interpretation

There are several possible explanations for the association of lower GABA concentration with the risk of postoperative delirium. First, a lower GABA concentration might be a sign of the severity of illness. In inflammatory states, glutamine concentrations in plasma and tissues are decreased due to many disease-related factors,[20] and it has also been reported that an increased severity of illness is a significant precipitating factor for postoperative delirium.[21] Second, administration of a sedative agent, such as propofol, for patients needing MV might lower the GABA concentration and contribute to an increased risk of delirium.[22] Propofol has been shown to positively modulate GABA type A receptor function,[23] which has interplayed with plasma GABA concentration. Third, abnormal GABA synthesis or metabolism in the postoperative period might trigger postoperative GABA activity changes and subsequent lower the GABA concentration, which may in turn contribute to the development of delirium. GABA is one of the most important inhibitory transmitters in the central nervous system. Alteration of GABA activity was reported in patients being treated with benzodiazepines, patients undergoing alcohol withdrawal and patients with cirrhosis,[7-9] and many studies have shown that these patients had a significantly higher risk of delirium.[10-12] These facts suggest a possible link between alterations of GABA activity and delirium. It should be noted that a lower GABA concentration was independently related to the risk of delirium even after adjustment for the severity of illness and the requirement for MV. Finally, there might be another unknown mechanism or a combination of mechanisms.

Although our study showed just the association, our current finding might be relevant to understanding the possible link of the GABA agonist with the incidence of delirium, and the effect of limiting the use of sedatives (especially benzodiazepines) in postoperative setting.[24,25]

Conclusions

In postoperative patients needing intensive care for 48 hours, the plasma GABA concentration on POD 2 was significantly lower in patients with delirium than in those without delirium. Further study is needed to confirm or refute our findings.

Competing interests

None declared.

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Appendix. Standard anaesthesia procedure and decision protocol for discharge from the intensive care unit

- Anaesthesia was induced with propofol (1–2 mg/kg), rocuronium (0.6 mg/kg) and fentanyl (1–2 μg/kg) or remifentanil (0.3–0.5 μg/kg/minute) to facilitate tracheal intubation.
  Anaesthesia was maintained by sevoflurane inhalation or propofol infusion with fentanyl and/or remifentanil.
  When epidural anaesthesia was used, the epidural catheter was inserted preoperatively and a pre-emptive dose of local anaesthetic was given before the surgical incision.
  Patients without epidural anaesthesia received an intravenous, patient-controlled morphine infusion, programmed to deliver morphine boluses of 1 mg with a lockout time of 10 minutes.
- The decision for discharge from the ICU was made by attending doctors who were blinded to the results of confusion assessment method in the intensive care unit tests, and to the plasma gamma-aminobutyric acid concentrations, when a patient's physiological status had stabilised.
  Patients discharged from the ICU within 48 hours were excluded from our study.