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Effect of Flumazenil on Bispectral Index Monitoring in Unpremedicated Patients

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Background: **Flumazenil is an imidazobenzodiazepine that promptly reverses *via* competitive inhibition the hypnotic/sedative effects of benzodiazepines on γ-aminobutyric acid receptors. Endogenous benzodiazepine ligands (endozepines) were isolated in urine, cerebrospinal fluid, and breast milk of women who had not received benzodiazepines. The bispectral index (BIS), an electroencephalographically derived parameter widely used for monitoring the effects of anesthetic/hypnotic drugs, was shown to correlate to various conditions that could influence electroencephalography. The authors examined the hypothesis that 0.5 mg of flumazenil administered to healthy unpremedicated patients during deep surgical remifentanil/propofol anesthesia would increase the BIS value and might expedite recovery from anesthesia.**

Methods: **Sixty healthy unpremedicated patients were randomly allocated to the flumazenil or control groups. After study drug administration, the authors compared BIS values and various recovery parameters in the flumazenil and control groups.**

Results: **BIS baseline values in the flumazenil group (38.7 ± 3.8) increased 15 min after flumazenil administration (53.2 ± 4.7), with a significant difference over time ($P < 0.0001$) between the two groups. Mean recovery parameters time, comprising time to spontaneous breathing, eye opening/hand squeezing on verbal command, extubation, and date of birth recollection, was significantly shorter ($P = 0.0002$) in the flumazenil group (6.9 ± 2.6 min) compared with the control group (9.8 ± 2.9 min).**

Conclusions: **This study demonstrates that flumazenil given to healthy unpremedicated patients during propofol/remifentanil anesthesia significantly increased the BIS value and allowed earlier emergence from anesthesia. This may indicate that flumazenil could be used on a case-by-case basis to reverse endogenous or exogenous endozepines that might play a role during anesthesia.**

FLUMAZENIL is an imidazobenzodiazepine that promptly reverses *via* competitive inhibition the hypnotic/sedative effects of benzodiazepines on γ-aminobutyric acid receptors.[1] Flumazenil was shown to have a beneficial analeptic stimulant effect in hepatic encephalopathy patients; a recent meta-analysis[2] of several double-blind randomized placebo-controlled trials described improvement in electroencephalographic signs of hepatic encephalopathy after flumazenil administration in patients who had not received sedative/hypnotic medications.[2] Endogenous benzodiazepines ligands (endozepines)[3] were isolated in urine,[4] cerebrospinal fluid,[5] and breast milk[6] of women who had not received benzodiazepines. It has been suggested that there might be a beneficial analeptic stimulant effect as a result of flumazenil's displacement of these endogenous benzodiazepine ligands.

The bispectral index (BIS), an electroencephalographically derived parameter widely used for monitoring the effects of anesthetic/hypnotic drugs, was shown to correlate to various conditions that could influence electroencephalography.[7] Meanwhile, there is paucity of information regarding the effects of central nervous system (CNS) stimulants on BIS monitoring when they are added to general anesthesia. Because flumazenil is a readily available medication that might exert a stimulant effect expediting anesthesia recovery, we tested the hypothesis that flumazenil administration in unpremedicated patients during steady-state deep surgical anesthesia would lead to an increase in BIS value as a surrogate parameter suggesting a lighter plane of anesthesia. Our primary endpoint was the effect of flumazenil administration on deep surgical propofol/remifentanil total IV anesthesia of BIS around 40. The secondary endpoints were the differences between the two groups in various anesthesia recovery parameters.

Materials and Methods

Our report of a prospective clinical consecutive randomized study was prepared in conformity with the guidelines of the consolidated standards of reporting trials (CONSORT) statement.[8] After approval by the Peking University First Hospital (Beijing, People's Republic of China) ethics committee, all patients who agreed to participate in the study gave written informed consent. Exclusion criteria were body mass index less than 20 or greater than 26 kg/m^2, treatment with cardiovascular or sedative/hypnotic drugs that might affect BIS monitoring,[7] and medical conditions that could affect the level of consciousness such as stroke, stupor, or dementia.

Using a computer-generated program, 60 unpremedicated patients with American Society of Anesthesiologists classification I-II undergoing upper/lower extremities or general surgical procedures of around 3 h with expected blood loss of less than 1 l were randomly allocated to the flumazenil or control groups. A BIS Quatro sensor (Aspect Medical Systems, Newton, MA)

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was placed on the patients' forehead according to manufacturer's recommendations and connected to a BIS-XP monitor (version 3.4; Aspect Medical Systems). Data from the BIS and electromyography power displayed in decibel (dB) units were continuously collected and stored every 5 s on a laptop computer, and the BIS smoothing window was set at 30 s. Recordings were started after verifying a signal quality index of greater than 95% and electrodes impedance of less than 5 kΩ.

The expected surgical procedure duration was around 3 h; therefore, the short-acting opioid fentanyl (1.5 μg/kg) was used for induction to avoid hypotension that might result from using remifentanil for induction.[9] Propofol target-controlled infusion (TCI) using a Diprifusor infusion pump (AstraZeneca, Macclesfield, United Kingdom) incorporating a three-compartment Marsh pharmacokinetic model[10] was started after entering patients' anthropometric data. Propofol TCI was set to reach a plasma concentration of 3 μg/ml over a period of 2 min, during which patients were allowed to breathe spontaneously *via* a facemask. Time to loss of consciousness as patients lost their eyelash reflex and no longer responded to verbal command, propofol TCI, and BIS values at loss of consciousness were recorded. Remifentanil (0.1–0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) continuous infusion was used for maintenance. Neuromuscular block at the adductor pollicis muscle was evaluated using train-of-four (TOF)–Watch (Organon, Oss, The Netherlands). The acceleration piezo-transducer of the TOF-Watch was attached to the thumb, and the ulnar nerve was stimulated supramaximally at the wrist (pulse width, 200 μs, square wave) *via* surface electrodes with TOF stimuli (2 Hz for 2 s) at 12-s intervals. T_1 (first twitch of the TOF) expressed as percentage of control response and the TOF ratio (T_4:T_1) were used for the evaluation of neuromuscular block. Rocuronium (600 μg/kg) was administered for tracheal intubation followed by 200 μg/kg rocuronium top-up doses maintaining T_1 at 25%. The lungs were mechanically ventilated with 40% oxygen in air and adjusted to maintain 30–40 mmHg end-tidal carbon dioxide. Patients were warmed using a forced-hot-air-blanket to maintain esophageal core temperature greater than 36°C. Patient fluid requirements were replaced with crystalloid solutions. Blood loss, estimated from swab/drape weighing and suction bottles, was replaced by 6% hydroxyethyl starch 130/0.4. Because exogenous catecholamines were shown to evoke changes in BIS readings,[11,12] patients who required exogenous catecholamine administration such as ephedrine or phenylephrine were excluded from the study. A stable BIS 40 for deep surgical anesthesia[13] was maintained with propofol TCI ± 0.2 μg/ml rate adjustments.

An assigned anesthesia nurse, the only one with access to the randomization code, prepared 0.5 mg of flumazenil or saline in identical syringes. All anesthesiologists were blinded to the group allocation. Forty-five minutes before the expected completion of the surgical procedure, remifentanil infusion was kept constant in the two groups at 0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$, corresponding to remifentanil 3.75 ηg/ml plasma TCI.[13] Patients were allowed to recover spontaneously from neuromuscular block up to 0.9 TOF ratio. Thirty minutes before the expected completion of the surgical procedure, a blinded research assistant administered the study drug. Mean arterial pressure and heart rate were recorded before and after study drug administration and during recovery from anesthesia. After discontinuation of anesthesia, a dedicated blinded anesthesiologist used a uniform, one verbal command every 20 s method to assess times to spontaneous respiration, eye opening/hand squeezing on verbal command, extubation, and date of birth recollection.

Statistical Analyses

The effect of flumazenil on BIS monitoring in unpremedicated patients was not previously examined; therefore, an *a priori* sample size power analysis was not possible. On the basis of the first 20 pilot patients in whom mean BIS increase after flumazenil administration was 11 ± 9 compared with a BIS increase of 3 ± 5 in placebo patients, we performed an interim power analysis *t* test ($\alpha = 0.05$); this showed that a group size of 19 patients would be required to reveal a statistically significant difference between the two groups with 90% power. We then increased our sample size to 30 patients to match other previously published studies of doxapram and aminophylline stimulant analeptic effects.[14,15] We used a two-way analysis of variance (ANOVA) model (group × time) to compare parameter differences between the two groups. Dunnett two-sided multiple-comparison *post hoc* test was used to compare BIS values at different time points. We used a simple model to compare recovery parameter differences between the two groups, namely the mean recovery parameters time, which comprised mean values of five recovery parameters (time to spontaneous breathing, eye opening/hand squeezing on verbal command, extubation, and date of birth recollection). Data were expressed as means ± SD, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using Number Crunching Statistical System 2007 (NCSS Inc., Kaysville, UT) and StatXact (Cytel Software Corporation, Cambridge, MA).

Results

There was no significant difference between the two groups in patient characteristics, time to loss of consciousness, propofol/BIS values at loss of consciousness, duration of surgical procedures, surgical blood loss, and propofol TCI requirements for stable BIS 40 (table 1).

Table 1. Patient Demographics, Propofol, and BIS Values at Loss of Consciousness, Loss of Consciousness Time, Duration of Surgical Procedure, Surgical Blood Loss, and Propofol TCI Requirements for Stable BIS 40

	Flumazenil Group	Control Group
Male/female	14/16	17/13
Age, yrs	46 ± 11	51 ± 10
Weight, kg	68 ± 12	70 ± 5
Height, cm	165 ± 8	169 ± 15
BMI	24.9 ± 3.9	24.6 ± 2.2
Propofol TCI at LOC, μg/ml	2.8 ± 0.3	2.6 ± 0.2
BIS at LOC	65 ± 5	69 ± 8
LOC time, min	1.8 ± 0.6	1.6 ± 0.5
Duration of surgical procedure, h	3.2 ± 1.1	2.9 ± 1.4
Surgical blood loss, ml	633 ± 191	548 ± 251
Propofol TCI for stable BIS 40, μg/ml	3.9 ± 0.2	4.1 ± 0.1
Estimated remifentanil TCI, ηg/ml	3.75	3.75

Data are means ± SD. BIS = bispectral index; BMI = body mass index; Estimated remifentanil TCI = estimated remifentanil TCI 45 min before the expected completion of surgery; LOC = loss of consciousness; TCI = target controlled infusion. $P > 0.05$ Student *t* test.

Electromyography throughout the recordings was below 35 dB. Data were normally distributed. Two-way ANOVA (group × time) revealed a significant difference ($P < 0.0001$) between the two groups over time in BIS values (fig. 1). The degree-of-freedom was 1, 14, 14, and the F ratio was 76.8, 98.2, and 53.8 for the group-, time-, and group-time-effect, respectively. Dunnett two-sided multiple-comparison *post hoc* test revealed a significant difference between the two groups starting 6 min after flumazenil administration. Mean recovery parameters time was significantly shorter ($P = 0.0002$) in the flumazenil group compared with the control group (table 2).

Although mean arterial pressure and heart rate before flumazenil administration (86 ± 10 mmHg, 64 ± 11) decreased slightly 15 min after flumazenil administration (82 ± 13 mmHg, 61 ± 8), there were no significant differences over time between the two groups. We encountered no serious cardiovascular events after flumazenil administration.

Table 2. Recovery Parameters

	Flumazenil Group	Control Group	*P*
Time (min) to			
Spontaneous breathing	4.6 ± 1.8	5.7 ± 2.1	0.0224
Eye opening on verbal command	5.4 ± 2.8	7.2 ± 3.8	0.0431
Hand squeezing on verbal command	6.4 ± 3.1	10.4 ± 2.9	0.0001
Extubation	8.7 ± 2.5	12.8 ± 3	0.0001
Date of birth recollection	9.8 ± 3.2	13.1 ± 3.1	0.0001
Mean recovery parameters time	6.9 ± 2.6	9.8 ± 2.9	0.0002

Data are means ± SD, n = 30. Mean recovery parameters time = mean value of five recovery parameters (spontaneous breathing, eye opening/hand squeezing on verbal command, extubation, and date of birth recollection).

Discussion

Our main finding that flumazenil administration in unpremedicated patients results in a significant increase in BIS value during propofol/remifentanil anesthesia could be attributed to flumazenil partially antagonizing the sedative effects of general anesthesia. Here we can speculate that, although propofol is not thought to bind to the benzodiazepine site on the γ-aminobutyric acid receptor, it seems possible that flumazenil could have interacted in some way with the γ-aminobutyric acid receptor to reverse the effect of propofol. Earlier report suggest that flumazenil lacks intrinsic activities; 0.3 mg of flumazenil administered after midazolam led to a prompt restoration of acoustical and somatosensory-evoked cortical responses in volunteers, but flumazenil administered without benzodiazepines did not change acoustical or somatosensory-evoked cortical responses.[16] Nevertheless, our observed BIS arousal effect could still

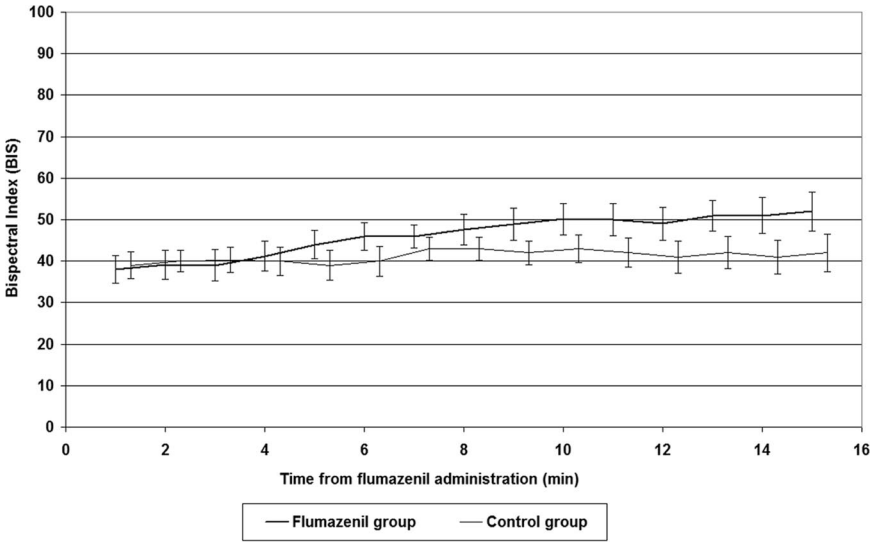

Fig. 1. Mean bispectral index (BIS) ± SD after flumazenil administration in the flumazenil and control groups (n = 30). Two-way ANOVA followed by *post hoc* testing revealed a significant difference between the two groups starting 6 min after flumazenil administration.

be attributed in part to an intrinsic direct CNS stimulant effect, as BIS values attained significant difference between-groups shortly after flumazenil administration. Although flumazenil has hitherto been clinically described as an agent with few intrinsic properties with a primary effect lying in its ability to reverse benzodiazepines, we have shown that flumazenil might possess some intrinsic CNS stimulant activity that could result in an enhanced arousal from general anesthesia.

Another hypothesis proposes flumazenil inhibition of γ-aminobutyric acid-benzodiazepine receptor complex of endogenous benzodiazepine-like ligands (endozepines) in patients who had not received benzodiazepines, thus resulting in a significant increase in BIS values. The discovery of high-affinity benzodiazepine binding sites in human cerebral cellular tissues[17] marked the beginning of a search for putative endogenous receptor ligands according to a review article by Sand *et al.*[18] Naturally occurring endozepines that did not result from environmental contamination with pharmaceutical benzodiazepines have been reported in different mammalian tissues.[19] Endogenous benzodiazepines in potentially physiologic concentrations were demonstrated in human cerebrospinal fluid,[5] serum,[20] plasma,[21] urine,[4] ultrafiltrates[4] of patients who had not received benzodiazepines, and breast milk of healthy, newly delivered women who were not taking benzodiazepines.[6,22] These comprised a variety of 1,4-benzodiazepines such as diazepam, *N*-desmethyldiazepam, oxazepam, and lorazepam that corresponded to commercially available drugs.[18] In 1990, proof was given of genuine benzodiazepines in human brains that had been conserved in paraffin since the 1940s, *i.e.*, well before the era of industrial benzodiazepine synthesis.[23]

The synthesis of naturally occurring benzodiazepines by vegetal cells had been originally suggested in 1987,[24] and it has been supported by recent compelling evidence from sterile cultures showing that wheat and potatoes could produce pharmacologically active benzodiazepine molecules during germination.[25,26] Moreover, several benzodiazepines that are not currently available for therapeutic use, such as deschlorodiazepam and isodiazepam, have been isolated from plants in support of a biosynthetic pathway, resulting in the *de novo* formation of benzodiazepines.[25] Natural benzodiazepines, such as delorazepam and temazepam, found in soil, plant, and animal tissues, are virtually indistinguishable from benzodiazepines of industrial origin in terms of chemical structure and pharmacological activity.[18] To date, the confirmation by mass spectrometric analyses[25,26] of the occurrence of at least nine different pharmacologically active natural 1,4-benzodiazepines in a number of plants and nutritive plant products commonly used for human consumption, such as potato tuber, wheat, rice, soy beans, cherries, maize, mushrooms, lentils, and grapes,[25,27,28] strongly suggests that these agents may be already incorporated in the food chain.[18] Although the levels of these "natural" endozepines might be quite low, reversing endogenous (or exogenous from food) benzodiazepine-like substances could be another possible mechanism.

We used BIS monitoring as a surrogate parameter suggesting a lighter plane of anesthesia after flumazenil administration. Likewise, previous studies have used BIS monitoring to quantify the analeptic effect of CNS stimulants. They demonstrated a significant increase of BIS values after doxapram/aminophylline administration during steady-state inhalational anesthesia.[14,15] This still raises the question of the real clinical relevance of our observations; analeptics or CNS stimulants are not commonly used in current anesthetic practice in the light of the predictable pharmacokinetic profile of modern anesthetics with short offset times. We believe that our study could still provide clinically relevant information regarding arousal by a readily available drug that could be used on a case-by-case basis in patients whose recovery from anesthesia is unexpectedly prolonged.

In our study, 45 min before the expected completion of surgical procedure, we kept remifentanil infusion constant in both groups at 0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$, corresponding to remifentanil 3.75 ηg/ml plasma TCI; this probably kept the two study groups as equal as is clinically possible. Remifentanil allows rapid recovery with a very short context-sensitive half time of 3–4 min independent of the duration of infusion or the total dose given.[29] Whereas opioids in the usual clinical doses were shown not to affect BIS monitoring; unlike intravenous or inhalational anesthetics, opioids in analgesic concentrations produce minimal or no electrophysiological alterations on the cerebral cortex.[30,31] Shafer and Varvel clearly demonstrated that opioid doses of almost five times the analgesic concentrations would be required for the appearance of a noticeable electroencephalographic depression[30] due to the fact that noncortical structures undetectable by the electroencephalograph, such as locus coeruleus-noradrenergic system, are involved in the mechanism of opioid drug effect.[31] As a result, the opioids we used in our study were unlikely to confound our results.

Electromyographic activities are artifact signals that occur within the frequency "range of interest" of the bispectrum and could simulate the BetaRatio, one of the BIS component descriptors that would be misinterpreted by the BIS algorithm as electroencephalographic activity.[32] In our study, electromyographic values were below what could be considered the cutoff value of 35 dB,[33] clearly indicating that high electromyographic activity did not confound the results of our study.

Our findings of a slight, nonsignificant decline in mean arterial pressure and heart rate after flumazenil administration concur with two previous double-blind, randomized placebo-controlled studies in which 0.2–0.5 mg of flumazenil administration reduced mean arterial pres-

sure by 3–5 mmHg and heart rate by 4–7 beats/min in healthy volunteers who did not receive benzodiazepines.[34,35] We encountered no serious cardiovascular events after flumazenil administration. Although ventricular arrhythmias have been reported with flumazenil administration in two benzodiazepine-overdose comatose patients,[36,37] we are unaware of any serious cardiovascular adverse events being reported with flumazenil administration in patients who had not received benzodiazepines.

Animal studies have shown that flumazenil administration could potentially increase cerebral blood flow and intracranial pressure during the reperfusion phase after incomplete global ischemia.[38] This could represent a serious limitation for the use of flumazenil in patients with compromised intracranial physiology. However, the design of our study that included only patients without neurologic conditions did not allow us to explore such an effect.

In conclusion, flumazenil administration during propofol/remifentanil anesthesia in healthy unpremedicated patients significantly increased the BIS value and allowed earlier emergence from anesthesia. This may indicate that flumazenil, on a case-by-case basis, could be useful for reversing endogenous or exogenous endozepines that might play a role during anesthesia.

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