Research Protocol

Examining the Variability in Adrenal Cortical Suppression Among Patients Receiving Etomidate for General Anesthesia in Morning vs. Evening Sessions: A Randomized Clinical Trial

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1. Rationale & background information：

Etomidate is a widely used intravenous anesthetic that primarily exerts its effects by acting on γ-aminobutyric acid (GABA) receptors in the central nervous system[1]. Its exceptional hemodynamic stability has established its pivotal role in the induction of general anesthesia[2]. Recent studies have demonstrated its non-inferiority to propofol for anesthetic maintenance, with certain potential advantages[3,4].

The suppressive effect of etomidate on adrenocortical function remains a major clinical concern[5-8]. Previous studies have demonstrated that continuous etomidate infusion induces transient adrenocortical suppression in elderly patients undergoing elective abdominal surgery, without significantly increasing the incidence of major in-hospital complications[3]. Notably, the hypothalamic-pituitary-adrenal (HPA) axis exhibits a pronounced circadian rhythm, with adrenocortical hormone concentrations fluctuating by up to several dozen-fold throughout the day[9]. However, it remains unclear whether this physiological circadian variation modulates etomidate-induced adrenocortical suppression and consequently affects postoperative recovery. Clarifying this issue is of particular importance in populous countries, where elective surgeries are frequently performed in the evening or at night. The safety of etomidate for anesthesia induction and maintenance during these time periods requires further investigation.

We hypothesized that etomidate's adrenal suppression is more pronounced in the evening than in the morning, leading to a prolonged recovery of adrenal corticosteroid concentrations and potential effects on postoperative recovery quality.

2. Objective：

Primary Aim

To investigate the differences in the inhibitory effects of etomidate on adrenal cortical function when administered in the morning versus the evening.

A. To compare the immediate postoperative changes in adrenocortical hormone levels from preoperative baseline between patients receiving sustained etomidate administration during morning versus afternoon surgical procedures.

B. To compare the two patient groups regarding the recovery of cortisol, aldosterone, and ACTH levels into the normal range on postoperative days 1 and 3.

Secondary Aims

To evaluate the impact of sustained etomidate administration in the morning and afternoon on postoperative recovery outcomes in patients.

3. Methods and study design

3.1 Study Overview

This study was a single center, randomized clinical trial.

3.2 Ethical considerations

Ethical approval for this study (Ethics Review No. (2024) Ethics Yan No. (09)) was provided by the Ethical Committee of the First Affiliated Hospital of Wannan Medical College, Wuhu, Chain (Chairman Prof Wu Pei) on 7 March 2024. And this study registered with the China Clinical Trial Registry (registration number: ChiCTR2400082034). All participants provided written informed consent prior to enrollment.

3.3 Setting

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3.4 Population

Inclusion criteria

1) Patients scheduled for abdominal surgery under general anesthesia

2) Aged 18 years or older

3) Expected duration of anesthesia: 2-4 hours

Exclusion criteria

1) ASA ≥3

2) A Body Mass Index (BMI) <18.5 kg $(m^2)^{-1}$ or >29.9 kg $(m^2)^{-1}$

3) Severe hepatic or renal dysfunction

4) A history of cerebrovascular accident, myocardial infarction, or unstable angina within the past 3 months

5) Preoperative blood pressure >180/100 mmHg

6) Concurrent endocrine disorders or a history of steroid therapy

7) Psychiatric illness or long-term use of psychotropic medications

8) Irregular work-rest schedules, including jet lag or frequent night shifts

9) Diabetic patients with complications

10) Confirmed or suspected abuse or long-term use of narcotic sedatives

11) Allergies to etomidate fat emulsion

12) Surgery within the past 3 months

13) Participation in another study within the past 30 days or refusal to participate

Withdrawal criteria

1) Duration of surgery > 4h or < 2h

2) Occurrence of severe intraoperative vital sign fluctuations

3) Postoperative transfer to the intensive care unit (ICU)

4) Preoperative cortisol, aldosterone, and ACTH concentrations outside the normal range

5) Loss to follow-up or patient request to withdraw from the trial

3.4 Intervention

Study groups and Randomization

To test this hypothesis, we studied patients undergoing surgery at two distinct time intervals: anesthesia initiation at 8:00–9:00 AM or 4:00–5:00 PM, with durations of 2–4 hours. Participants were randomized 1:1 using a random number table.

Group	Explanation
M	the Morning Group, anesthesia induction at 8:00–9:00 AM
E	the Evening Group, anesthesia induction at 4:00–5:00 PM

Research drugs

Name, Etomidate ; Manufacturer, Jiangsu Enhwa Pharmaceutical Co.; Formulation, 10 mL:20 mg

Administration

After the patients are randomized, the drugs will be administered according to the method.

Etomidate at a dose of 0.3 $mg \cdot kg^{-1}$ was used for the induction of anesthesia. An etomidate-propofol mixture (volume ratio 2:1) and remifentanil (0.1–0.5 $\mu g\ kg^{-1}\ min^{-1}$) were continuously infused to maintain a Bispectral Index (BIS) of 40–60.

3.5 Anesthesia protocol

Pre-anesthesia management

All patients enrolled in the trial were accommodated in single rooms, instructed to turn off lights at 9:00 PM on the night before surgery, and turn them on at 6:00 AM on the day of surgery. They adhered to fasting and fluid restriction requirements before surgery. Upon entering the operating room, peripheral veins were accessed, and lactated Ringer's solution was infused at a rate of 5 $ml\ kg^{-1}\ h^{-1}$. ECG, Pulse Oximetry (SpO_2), noninvasive blood pressure, temperature, and depth of anesthesia were routinely monitored, with invasive blood pressure monitoring conducted if necessary.

Anesthesia induction

A sequential intravenous bolus of 0.05 mg kg-1 midazolam, 0.5 μg kg-1 sufentanil, 0.3 mg kg-1 etomidate (Etomidate Fat Emulsion Injection, Nhwa Pharma, Xuzhou, China), and 0.2 mg kg-1 cisatracurium was administered. After achieving muscle relaxation, an endotracheal tube was placed using a video laryngoscope, and mechanical ventilation was

initiated to maintain an end-tidal partial pressure of carbon dioxide ($PETCO_2$) at 35–45 mmHg.

Anesthesia maintenance

An etomidate-propofol mixture (volume ratio 2:1) and remifentanil (0.1–0.5 μg kg^{-1} min^{-1}) were continuously infused to maintain a Bispectral Index (BIS) of 40–60. Cisatracurium (1–2 μg kg^{-1} min^{-1}) was administered for muscle relaxation. Intraoperative hypotension and bradycardia were managed using ephedrine and hyoscyamine. Muscle relaxants were discontinued approximately 30 minutes before the end of surgery, and 0.1 μg kg-1 sufentanil was administered for transitional analgesia, as well as 8 mg of ondansetron to prevent Postoperative Nausea and Vomiting (PONV). Anesthetic infusion was stopped at the conclusion of the procedure, and patients were transferred to the Post-Anesthesia Care Unit (PACU) for recovery. When tidal volume exceeded 6 ml/kg, respiratory rate stabilized at 13–20 bpm, and SpO_2 surpassed 90% with ambient air, the tracheal tube was removed. Patients were returned to the ward once their Aldrete score exceeded 9.

Postoperative care

Both groups received postoperative Patient-Controlled Intravenous Analgesia (PCIA), which included 100 μg sufentanil and 4 mg butorphanol. PONV were evaluated using a scoring system: mild (1–2),

moderate (3–6), and severe (7–10) symptoms. Pain was assessed using the Visual Analogue Scale (VAS), ranging from 0 (no pain) to 10 (worst possible pain). Quality of Recovery-15 (QoR-15) scores10 were recorded on the third postoperative day, and postoperative in-hospital complications were documented throughout the study.

Blood specimens

Patients provided 5 ml of venous blood from the unilateral elbow in a supine position at specified time points: preoperatively, immediately postoperatively, and at 8:00 AM on the first and third postoperative days. Samples were centrifuged at 3000 rpm for 10 minutes at 4°C to isolate 2 ml of serum, which was stored at -80°C until testing. Measure the concentrations of cortisol, aldosterone, and ACTH using appropriate instruments and corresponding reagents.

Testing Instruments and Reagents

Instruments/Reagents	Model	Manufacturer
Fully Automated Chemiluminescence Immunoanalyzer	MAGLUMI X8	Shenzhen New
Cortisol Assay Kit (Chemiluminescence Method)	130298003M	

Adrenocorticotropic Hormone (ACTH) Assay Kit (Chemiluminescence Method)	130298002M	Industries Biomedical Engineering Co., Ltd.
Aldosterone Assay Kit (Chemiluminescence Method)	130206007M	

Considering diurnal variations in adrenal corticosteroid hormones, normal serum reference ranges were as follows

Hormone	8–9 AM	4–5 PM
Cortisol	72.6–322.8 ng ml^{-1}	32.4–150.0 ng ml^{-1}
ACTH	11.27–80.81 pg ml^{-1}	3.0–30.0 pg ml^{-1}
Aldosterone,	30–160 pg ml^{-1}	

3.6 Outcomes

Primary endpoints

Adrenocortical function: Concentration of cortisol, aldosterone and ACTH concentrations preoperatively, immediately postoperatively, and at 8:00 AM on the first and third postoperative days

Secondary endpoints

1) Time to response to verbal command

2) Time to extubating the tracheal tube

3) Time to discharge from postanesthesia care unit (PACU)

4) Postoperative length of stay

5) To evaluate postoperative Nausea and Vomiting (PONV) score, using a scoring system: mild (1–2), moderate (3–6), and severe (7–10) symptoms

6) To evaluate pain score, using the Visual Analogue Scale (VAS)

7) Quality of Recovery-15 (QoR-15) scores

8) Postoperative in-hospital complications, including pulmonary-related, gastrointestinal-related, cardiovascular-related, urologic-related and wound infection.

3.7 Data Collection

This was an open-label study, where researchers, participants, and staff were aware of group assignments. However, subjective endpoints were evaluated by an independent, blinded assessor to ensure objectivity and accuracy. Cases that failed to meet study criteria, due to variations in operation duration or unforeseen circumstances, were excluded from analysis.

3.8 sample size calculation

The primary objective was to compare the degree of serum cortisol suppression between the M group and E group immediately postoperatively in relation to etomidate's inhibitory effects. Pre-experimental findings indicated that the degree of serum cortisol suppression {[(preoperative value - immediately postoperative value) / preoperative value] × 100%} was 32.5% ± 16.8% in the M group (n=12) and 41.1% ± 17.0% in the E group (n=12). Using GPower 3.1.9.211 software with a power of 80% and an alpha level of 0.05, the minimum required sample size per group was calculated to be 62 patients, accounting for a 20% dropout rate. Thus, 78 patients per group were required, for a total of 156 participants.

3.9 Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA) and SPSS 20 (IBM Corporation, Armonk, NY, USA) software. Normally distributed data were expressed as mean ± SD and analyzed with Student's t-test. Non-normally distributed data were presented as median [IQR, range] and compared using Wilcoxon's test. Categorical data were expressed as n (%), and comparisons were made using the Chi-square test. The correlation between fluctuations in cortisol, aldosterone, and ACTH and the total etomidate dose was analyzed using Spearman's rank correlation test. A P-value < 0.05 was considered statistically significant.

Funding:

This study was supported by funding from Anhui University Research Project (No. 2023AH040252, 2023AH051745), Wuhu Science and Technology Plan Project (Grant No. 2024kj049), the Key Project of Anhui Province 'Leading the Charge with Open Competition', and Anhui Province clinical key specialty project fund.

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