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Effect of Remimazolam Combined with Propofol Anesthesia on Perioperative Cellular Immunity in Patients Undergoing Laparoscopic Ovarian Cyst Surgery

Xintao Dai[1], Zhenfeng Cai[1], Xingli Jiang[2] and Pinglei Wu[1]*

[1]*Department of Anaesthesiology, The People's Hospital of Yuhuan, Yuhuan, 317600, China*
[2]*Department of General Surgery, The People's Hospital of Yuhuan, Yuhuan 317600, China*

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XD and ZC conducted the experiments in this study. XJ and PW contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

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Laparoscopic surgery, Ovarian cyst, Remimazolam, Propofol, Immune cells

ABSTRACT

The aim of this study is to investigate the effects of remimazolam and propofol anesthesia on perioperative immune cell levels in patients undergoing laparoscopic ovarian cyst surgery (LOCS). The sample of 90 LOCS patients was randomly divided to an experimental group (EG) and a control group (CG) (45 per group). Participants in the CG were operated under propofol anesthesia, while in the EG it was under remimazolam anesthesia on the basis of the CG. The levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and blood oxygen saturation (SpO2) in the CG were lower than those in the EG during and after anesthesia. After 72 h, SAS in the EG was lower than those in the CG. Compared with the effect of propofol alone, the implementation of remimazolam anesthesia in LOCS patients was significant and could shorten the time of anesthesia-related indicators, and the level of vital sign changes during anesthesia was relatively more stable and had a smaller effect on the perioperative cellular immune level.

INTRODUCTION

Ovarian cysts are common gynecological conditions, mainly pathological cystic structures that grow inside or on the surface of the ovary. Data surveys show that the annual incidence of the disease can reach 23.9% and has a high prevalence among women of reproductive age (Bould *et al*., 2022). Laparoscopic ovarian cyst surgery (LOCS) is an important treatment for ovarian cysts, removing the lesion through minimally invasive treatment, with the advantages of small incision and quick postoperative recovery.

Although the effect of surgical treatment is stable, it requires the establishment of carbon dioxide pneumoperitoneum during surgery, which makes patients vulnerable to fluctuations in vital signs and immune cells are prone to immune escape under the action and stimulation of anesthetic drugs, leading to aggravation of the disease. This makes complications and reduce the surgical outcome (Lalonde, 2022). With the widespread use of minimally invasive clinical techniques, laparoscopic techniques have been rapidly developed and applied in the treatment of ovarian cysts, improving the condition while reducing the occurrence of postoperative complications and promoting early recovery from the disease (Romeo *et al*., 2021).

Therefore, the selection of appropriate anesthetic drugs in patients undergoing LOCS is important to stabilize patients vital signs, reduce immunosuppression, and improve the anesthetic effect. Propofol is a drug commonly used in surgical anesthesia, with the advantages of rapid induction of anesthesia and rapid postoperative recovery, but the drug tends to depress the circulation and respiration, causing anesthetic drug syndrome (Dauwe *et al*., 2021; Chen *et al*., 2022). However, remimazolam is a new type of drug in anesthesia, which has the advantages of rapid onset of action, short maintenance and recovery, and the time of drug infusion does not affect its time-

* Corresponding author: wupinglei1974@163.com
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volume-related half-life, and the pharmacokinetic changes show a linear trend, which is efficient and safe in general anesthesia (Abdelsalam, 2021). Yeung *et al*. (2021) showed that the implementation of remimazolam combined with propofol anesthesia in patients undergoing laparoscopic partial hepatectomy significantly improved the anesthetic effect, stabilized the level of vital signs, and reduced the adverse effects on immune cells. At present, there are few studies on the application of remimazolam combined with propofol in patients undergoing LOCS at home and abroad. In order to analyze the effect of anesthesia implementation, this study selected patients undergoing LOCS as the study object, aiming to select appropriate anesthetic drugs and improve the anesthetic effect, which is reported below.

MATERIALS AND METHODS

Subjects

Ninety patients who underwent LOCS at the institution were selected for implementation of the survey. Patients in both groups were eligible to join the study if they met the following inclusion criteria: (1) Postoperative pathological examination confirmed the diagnosis of ovarian cysts, and the pathological types included chocolate cysts, epithelial cysts, and teratomas. (2) First time cyst dissection was performed. (3) American Society of Anesthesiologists (ASA) classification I-II; (4) Cyst diameter >5 cm; (5) Normal mental status, cognitive function, and audiovisual function, and can cooperate with the surgical implementation. Patients with the following criteria were excluded from the study: (1) Malignant lesions of ovarian cysts. (2) Previous treatment with hormonal drugs. (3) Previous history of severe allergy to anesthetic drugs. (4) Combination of more serious endocrine diseases, such as diabetes mellitus, hyperthyroidism, hypothyroidism. (5) Patients with combined insulin resistance. Samples are categorized as the control group (CG, n=45) and the experimental group (EG, n= 45) randomly.

Anesthesia method

After the preoperative preparation and admission to the operating room, the patient's vital signs were monitored by connecting the cardiac monitoring equipment, and an effective intravenous access was established after selecting a suitable vein and giving 2~4L/min oxygen mask inhalation to start the implementation of intravenous anesthesia.

Anesthesia was administred to both groups. The anesthetic drugs were selected for intravenous injection in sequential order, and the drugs applied sequentially included 1.5~2.5 mg/kg of propofol, 0.4~0.5 μg/kg of Sufentanil, and 0.15~0.2mg/kg of Cisatracurium. Patients were observed for muscle relaxation, and if the criteria were met, tracheal intubation was implemented and mechanical ventilation was performed to maintain normal breathing.

In the CG, 4~12mg/(kg-h) of propofol drug was used to maintain anesthesia by infusion with an intravenous infusion pump. In the EG, 0.4~1.2mg/(kg-h) of remimazolam benzoate was administered intravenously. Meanwhile, remifentanil was pumped into the vein at a rate of 0.2~0.5 μg/kg (kg-min) in both groups, and 0.03 mg/kg cisatracurium was injected every 20 min intraoperatively to maintain the muscle relaxation state.

Clinical information data

The study used a self-administered general information questionnaire, including patients age, weight, cyst site, disease type, tumor diameter, etc. The questionnaire was distributed on the spot, and the patients were instructed to fill out the contents of the questionnaire within the specified time of 30 min. The questionnaire was collected and checked for omissions and errors, and they were asked to add them in time.

Anesthesia effect index

The anesthesiologist cooperated with the investigator to collect the onset time of anesthesia, disorientation recovery time, eyelash reflex time, and awakening time of the patients after the operation (Levin and Meyer, 2021).

Vital sign levels are tested during and after anesthesia, respectively. The investigator was assisted by a visiting nurse to collect and record the patient's systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and blood oxygen pulse oxygen saturation (SpO_2). The normal values of each index level were SBP: 90-120 mmHg; DBP: 60-90 mmHg; HR: 60-100 beats/min; SpO_2: >95% (Jensen *et al*., 2022).

Immune cytokine levels

The attending physician assisted the investigator to collect $CD3^+$, $CD4^+$, CD^+, $CD4^+/CD8^+$, NK cell levels by laboratory tests. The BD Trucount tube was added with the appropriate cellular antibodies and anticoagulant of the lymphocyte subpopulation kit, which was mixed thoroughly and left for 30 min protected from light. Then 2 ml of hemolysin was mixed in it and left for 30 min at room temperature between 20 and 25°C for testing (Suryawanshi *et al*., 2022).

Adverse reactions and physical health status

Propofol anesthesia's most common adverse reactions include agitation, delirium, nausea and vomiting, and hypoxemia. The adverse reactions were also used as

evaluation indicators in this study. Relevant information was obtained by daily questioning and recording of sample patients by designated medical staff. The SAS was administered once before the start of surgery and once 72 h after surgery. SAS scores before and after surgery in the same group, as well as between different groups, were used for comparative studies.

Statistical analysis

Data were entered into the software SPSS 26.0 for analysis and processing. The number of cases (n) and rate (%) describes the count data, and the χ^2 test was performed between groups; the mean ± standard deviation describes the measurement data conforming to normal distribution, and the independent samples t-test was performed between groups. The differences of data within groups were compared using repeated measures ANOVA.

RESULTS

The results for the anesthesia outcome for anesthesia onset time, fixation recovery time, lash firing time, and awakening time show in Figure 1. Patients in the EG significantly reported lower symptom levels on anesthesia onset time, fixation recovery time, lash firing time, and awakening time than the CG.

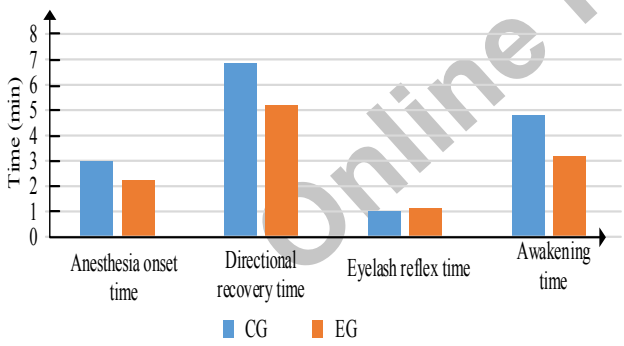

Fig. 1. Comparison of patient anesthesia outcomes.

Table I indicates descriptive data for the vital sign and immune cell outcomes during anesthesia. Before anesthesia, no statistically significant differences found in the vital signs level between EG and CG. There were statistically significant differences in the levels of SBP, DBP, HR, and SpO_2 in CG ($P<0.05$). There were no statistically significant differences in the levels of SBP, DBP, HR, and SpO_2 in EG ($P> 0.05$). The levels of SBP, DBP, HR, and SpO_2 were higher in EG than in CG ($P<0.05$).

Table I. Anesthesia outcome scores for the patients in the study groups.

Indicator	Anesthesia time	Group (x±s)		X^2/t	P
		CG (n=45)	EG (n=45)		
SBP (mmHg)	Before	125.37±11.29	125.40±12.07	0.012	0.990
	Under	120.58±9.37	126.49±11.67	2.649	0.010
	After	121.64±10.69	126.96±13.08	2.113	0.038
DBP (mmHg)	Before	58.34±11.06	58.40±10.68	0.026	0.979
	Under	52.11±10.16	56.89±11.07	2.134	0.036
	After	50.34±9.67	56.62±11.37	2.822	0.006
HR (times/ min)	Before	75.28±7.16	75.49±7.23	0.138	0.890
	Under	66.67±9.25	73.03±9.40	3.235	0.002
	After	69.66±10.37	75.19±10.64	2.497	0.014
SpO2 (%)	Before	96.48±3.45	96.55±3.28	0.324	0.747
	Under	94.27±2.16	95.19±3.01	1.666	0.099
	After	93.99±2.77	95.64±2.82	2.800	0.000
$CD3^+$ (%)	Before	75.18±6.41	75.23±6.37	0.037	0.971
	Under	56.88±6.27	68.49±7.02	8.274	0.000
	After	74.18±5.04	74.26±5.12	0.075	0.941
$CD4^+$ (%)	Before	44.69±6.37	44.71±6.52	0.015	0.988
	Under	29.08±5.17	39.36±5.85	8.833	0.000
	After	43.29±6.07	43.04±6.25	0.192	0.848
NK cells (%)	Before	18.48±3.48	18.61±3.29	0.182	0.856
	Under	11.75±2.69	16.27±2.16	8.789	0.000
	After	17.75±2.11	18.36±2.13	1.365	0.176
$CD4^+$ / $CD8^+$	Before	1.37±0.49	1.41±0.36	0.441	0.660
	Under	0.79±0.31	1.18±0.45	4.788	0..000
	After	1.39±0.28	1.31±0.37	1.57	0.251

SBP, Systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SpO_2, blood oxygen puluse oxygen saturation; CD, ...?; NK, ...?

At 24h postoperatively, the levels of $CD3^+$, $CD4^+$, NK cells, $CD4^+/CD8^+$ in both groups were lower than 30 min before induction of anesthesia, and the levels of NK cells in EG were higher than CG at any time ($P<0.05$). The levels of $CD3^+$, $CD4^+$, NK cells, $CD4^+/CD8^+$ at 72h postoperatively were not statistically significant compared with those before surgery ($P>0.05$); compared with 24h postoperatively, the levels of $CD3^+$, $CD4^+$, NK cells, $CD4^+/CD8^+$ were higher at 72 h postoperatively ($P<0.05$) (Table I).

The occurrence of adverse reactions during the awakening period for the two groups of patients showed significant differences. No statistically significant

differences were found between the two groups for any of the four major adverse reactions (Table II). In terms of psychological indicators, no significant differences were seen between the two groups in terms of SAS scores before and 24 h after surgery, while 72 h after surgery showed lower EG scores than CG (Table III).

Table II. Occurrence of adverse reactions during the awakening period in both groups.

Group	Restlessness	Delirium	Nausea and vomiting	Hypoxemia
CG	8	4	4	1
EG	6	4	3	1
P	0.163	0.389	0.147	0.514

Table III. The difference in SAS between the two groups before and after surgery.

Group	Before	72h after
CG	4.8±0.5	3.2±0.7
EG	4.7±0.3	3.6±0.5
P	0.071	0.001
F	2.03	14.51

DISCUSSION

The implementation of remimazolam combined with propofol anesthesia shortened the onset of anesthesia, recovery time of fixation, lash reflex time, and awakening time in patients undergoing LOCS compared with propofol anesthesia alone. Propofol mainly acts on the $GABA_A$ receptor in the locus coeruleus, inhibiting noradrenergic neurons, and ensuring a gradual decrease in the release of norepinephrine from the prefrontal sebum. After drug anesthesia, it can also act on the $GABA_A$ receptors in the body's cerebral cortex and the ventrolateral nucleus of the preoptic region of the hypothalamus, interfering with the neural synaptic function and affecting the corresponding activities of the cerebral cortex, thereby causing the patient to lose consciousness before surgery, achieving the purpose of anesthesia (Mohammed *et al*., 2021). However, the administration of the drug alone will contribute to an increase in chloride conduction and decrease peripheral vascular resistance, which in turn is consistent with the circulatory system, with a high incidence of adverse reactions. In contrast, remimazolam, a benzodiazepine, has significant sedative, hypnotic, and anxiolytic effects on the body, and can raise the patient's wakefulness threshold and shorten the duration of sleep induction after anesthesia. Silva *et al*. (2021) studied remimazolam in disease sedation and induction of general anesthesia. The new remimazolam is a new generation of anesthetic drugs with rapid metabolism and no significant pharmacological activity of metabolites by introducing side chains in the benzodiazepine ring. Remimazolam combined with propofol can be applied to the same receptor, and the anesthetic effect can be synergistic, which not only reduces the dose of anesthesia alone, but also significantly reduces the adverse effects of drugs, improves the effect of anesthesia, shortens the onset of anesthesia, recovery time of fixation, lash reflex time, and awakening time (Marra *et al*., 2021).

At each time point, patients undergoing propofol anesthesia alone had greater fluctuations in SBP, DBP, HR, and SpO_2 levels under the influence of anesthetic drugs, while patients undergoing combined remimazolam combined with propofol anesthesia had less fluctuations in SBP, DBP, HR, and SpO_2 levels under the influence of anesthetic drugs. This indicates that the joint implementation of anesthesia has less impact on changes in vital sign levels and is more conducive to a stable state of perioperative vital sign levels. Remimazolam has a high clearance rate in application and does not accumulate in the body after intravenous anesthesia, and also has a strong central inhibitory effect, which exerts anesthetic effects to reduce the patient's stress response during surgery, ensuring sedation and anesthesia at the same time, and control the effect of drug inhibition of the body's circulatory system. After the combination of propofol to implement anesthesia, it enhances the pharmacological effect of the two drugs, reduces the amount of propofol, further reduces the inhibitory effect of drugs on the circulatory system, thus avoiding large fluctuations in blood pressure, heart rate and oxygen saturation in the perioperative period (Pipil *et al*., 2022).

The results of this study showed that 24 h postoperatively, $CD3^+$, $CD4^+$ in both groups were lower than 30min before anesthesia induction, and the level of each cell of EG was higher than CG. 72 h postoperatively, $CD3^+$, $CD4^+$ were insignificant compared with preoperative ($P>0.05$); compared with 24 h postoperatively, CD3+, CD4+, CD4+/CD8+ were higher at 72 h postoperatively ($P<0.05$). This indicates that the combined administration of remimazolam combined with propofol anesthesia has a less suppressive effect on immune cells compared to propofol anesthesia treatment alone. Lymphocytes such as $CD3^+$, $CD4^+$, $CD8^+$ have an important role in the normal circulation of the body's immune system, $CD3^+$ is mainly distributed in mature T cells, indicating the overall level of the body's cellular immunity, CD4 , $CD8^+$, $CD4^+$, and CD8 are helper T-lymphocytes and cytotoxic T-lymphocytes, respectively, and the two types of cells can be mutually regulated and coordinated, and a decrease in their levels

indicates that the immune function is suppressed under the influence of extrinsic factors. However, an increase in the level of immune cells activates the immune response and helps the immune system to maintain homeostasis, as well as to remove harmful substances from the body and maintain the stability of the internal environment (Petre *et al*., 2021). The application of propofol anesthesia alone, and remimazolam combined with propofol both produced suppression of immune cells, but the combination of anesthesia with propofol produced less suppression of immunity. Propofol has a suppressive effect on immunity, which may be related to the activation of the sympathetic nerve and HPA axis, which increases the secretion of cortisol in the body. This affects the immune function through the inhibition of prostaglandins or the release of reactive substances during the acute phase of the disease. There is conclusion that benzodiazepines have significant anti-inflammatory effects on the immune system, as well as inhibiting the elevated levels of cortisol and adrenocorticotropic hormones during the stress response (Ke *et al*., 2022). Remimazolam is a new type of benzodiazepine, which can suppress cortisol and adrenocorticotropic hormone levels during surgery and provide effective protection of immune function. Both drugs act on the body by activating the central $GABA_A$ receptors, which act as mediators of the immune effects of the drugs.

According to the experimental results, remimazolam combined with propofol anesthesia did not make a significant difference in adverse reactions in patients undergoing LOCS compared to propofol-only drugs. In terms of the patients' postoperative mental health, there was no statistically significant difference in the preoperative SAS scores between the two groups. The SAS test at 72 h postoperatively, on the other hand, showed significantly lower SAS scores in the experimental group than in the CG patients. This indicates that patients who received remimazolam combined with propofol anesthesia were able to recover a healthy mental state more quickly. The mental health of patients after surgery is influenced by a combination of factors, so the effect of both anesthesia methods on patients postoperative anxiety and other symptoms needs to be further investigated, but this result has tentatively indicated the value of the application of remimazolam combined with propofol anesthesia.

There are some limitations in the study. The sample selected for the study was a single center, the sample size was small and the age range was small, for this reason, the sample size of the study should be increased in multiple centers and the age of the study should be expanded to further explore and complete the study results.

CONCLUSION

The effect of remimazolam combined with propofol anesthesia in patients undergoing LOCS was better than that of propofol anesthesia alone, and the drug onset time, fixation recovery time, lash reflex time, and awakening time were shortened, and the vital signs were at normal levels during anesthesia, and the combined anesthesia had less effect on them and less effect on immunosuppression. The effect of combined anesthesia on the immunosuppression was less severe.

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IRB approval

This study was approved by the Advanced Studies Research Board of the People's Hospital of Yuhuan, Yuhuan, China.

Ethical approval

The study was carried out in compliance with guidelines issued by the hospital ethics committee of the People's Hospital of Yuhuan, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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