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REPORTS OF ORIGINAL INVESTIGATIONS

The immunomodulatory effect of ketamine in colorectal cancer surgery: a randomized-controlled trial

Effet immunomodulateur de la kétamine lors d'une chirurgie de cancer colorectal : une étude randomisée contrôlée

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Abstract

Purpose *Ketamine's inhibitory action on the N-methyl-D-aspartate receptor and anti-inflammatory effects may provide beneficial immunomodulation in cancer surgery. We investigated the effect of subanesthetic-dose ketamine as an adjunct to desflurane anesthesia on natural killer (NK) cell activity and inflammation in patients undergoing colorectal cancer surgery.*

Methods *A total of 100 patients were randomly assigned to a control or ketamine group. The ketamine group received a bolus of 0.25 mg·kg^{-1} ketamine five minutes before the start of surgery, followed by an infusion 0.05 mg·kg^{-1}·hr^{-1} until the end of surgery; the control group received a similar amount of normal saline. We measured NK cell activity and proinflammatory cytokines (interleukin-6 [IL-6] and tumour necrosis factor-α [TNF-α]) before surgery and one, 24, and 48 hr after surgery. C-reactive protein (CRP) was measured before surgery and one, three, and five days after surgery. Carcinoembryonic antigen and cancer recurrence/metastasis were assessed two years after surgery.*

Results *The NK cell activity was significantly decreased after surgery in both groups, but the change was not different between groups in the linear mixed model analysis (P = 0.47). Changes in IL-6, TNF-α, CRP, and carcinoembryonic antigen levels were not different between groups (P = 0.27, 0.69, 0.99, and 0.97, respectively). Cancer recurrence within 2 years after surgery was similar between groups (10% vs 8%, P = 0.62).*

Conclusions *Intraoperative low-dose ketamine administration did not convey any favourable impacts on overall postoperative NK cell activity, inflammatory responses, and prognosis in colorectal cancer surgery patients.*

Trial registration *www.clinicaltrial.gov (NCT03273231); registered 6 September 2017.*

Résumé

Objectif *L'action inhibitrice de la kétamine sur le récepteur du N-méthyle-D-aspartate et ses effets anti-inflammatoires pourraient procurer une immunomodulation bénéfique lors d'une chirurgie oncologique. Nous avons étudié l'effet de la kétamine en dose sous-anesthésique en complément à une anesthésie au desflurane sur l'activité des cellules tueuses naturelles (NK) et l'inflammation chez les patients subissant une chirurgie de cancer colorectal.*

Méthode *Au total, 100 patients ont été randomisés à un groupe témoin ou kétamine. Le groupe kétamine a reçu un bolus de 0,25 mg·kg^{-1} de kétamine cinq minutes avant le début de la chirurgie, suivi d'une perfusion de 0,05 mg·kg^{-1}·h^{-1} jusqu'à la fin de la chirurgie; le groupe*

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témoin a reçu une quantité similaire de solution physiologique salée. Nous avons mesuré l'activité des cellules NK et des cytokines pro-inflammatoires (interleukine-6 [IL-6] et facteur de nécrose tumorale α [TNF-α]) avant la chirurgie et une, 24 et 48 heures après la chirurgie. La protéîne C réactive (CRP) a été mesurée avant la chirurgie puis un, trois et cinq jours après la chirurgie. L'antigène carcinoembryonnaire et la récurrence du cancer ou les métastases ont été évalués deux ans après la chirurgie.

Résultats *L'activité des cellules NK a été significativement réduite après la chirurgie dans les deux groupes, mais le changement ne différait pas entre les groupes dans l'analyse de modèle mixte linéaire (P = 0,47). Les changements dans les taux d'IL-6, de TNF-α, de CRP, et d'antigène carcinoembryonnaire n'étaient pas différents entre les groupes (P = 0,27, 0,69, 0,99 et 0,97, respectivement). La récidive du cancer au cours des deux années suivant la chirurgie était similaire entre les groupes (10 % vs 8 %, P = 0,62).*

Conclusion *L'administration peropératoire de kétamine de faible dose ne s'est pas traduite par un quelconque impact favorable sur l'activité postopératoire g des cellules NK, la réaction inflammatoire, et le pronostic chez les patients de chirurgie de cancer colorectal.*

Enregistrement de l'étude *www.ClinicalTrials.gov (NCT03273231); enregistrée le 6 septembre 2017.*

Keywords ketamine · natural killer cell · colorectal cancer · cancer surgery · inflammation · immunity

For many solid tumours, surgery is the only curative treatment. Nevertheless, it is inevitably accompanied by stress responses that paradoxically increase the perioperative risk for the dissemination and metastasis of cancer cells.[1] Notably, suppression of natural killer (NK) cell activity, which constitutes the primary innate defense mechanism against cancer metastasis,[2] has been recognized as a key target of this surgical-induced immunomodulation.[3,4] Indeed, NK cell activity was significantly lower in patients with colorectal cancer than in healthy participants and was further suppressed by more than 80% from baseline following surgery.[5] Additionally, this activity has been shown to be a strong prognostic factor in colorectal adenocarcinoma.[6,7] Thus, emerging evidence supports the potential importance of anesthesia-related immunomodulation on cancer recurrence as a part of this perioperative stress response. Nevertheless, a definite association between various anesthetic agents and immune cell activity, cytokine secretion, and tumour cell behaviour remains unsettled.[8–10]

Ketamine is an *N*-methyl-D-aspartate (NMDA) receptor antagonist used for anesthesia and analgesic purposes. Ketamine in anesthetic or higher doses (up to 80 $mg \cdot kg^{-1}$) has been shown to suppress NK cell activity, possibly via sympathetic activation.[11] In addition, subanesthetic low-dose ketamine as an adjunct to general anesthesia reduced inflammatory responses[12] and pain after cancer surgery,[13] all of which could be advantageous for mitigating the suppression of NK cell activity. Furthermore, ketamine may exert a direct influence on NK cell activity as its suppression also involves the activation of NMDA receptors and subsequent changes in intracellular calcium and reactive oxygen species.[14] Nevertheless, no clinical evidence exists on the effect of low-dose ketamine on NK cell activity and inflammation in colorectal cancer surgery.

In this randomized-controlled trial, we assessed the effect of subanesthetic, low-dose ketamine on NK cell activity and inflammatory cytokine levels in patients undergoing colorectal cancer surgery. Based on previous experimental and clinical findings,[12–14] we hypothesized that ketamine would attenuate immunosuppression during the perioperative period in these patients.

Methods

This study was approved by the Institutional Review Board and Hospital Research Ethics Committee of the Severance Hospital, Yonsei University Health System, Seoul, Korea (#4-2017-0475) in July 2017 and registered at clinicaltrial.gov (NCT03273231) prior to patient enrolment. Patients aged 20–80 yr with American Society of Anesthesiologists physical status I–III and who were scheduled for elective laparoscopic colorectal cancer resection in the Severance Hospital were enrolled between 6 September 2017 and 11 April 2018. Patients were excluded if they met at least one of the following criteria: inflammation or infection symptoms, immune or endocrine disorders, immunosuppressive therapy, steroid administration within the last six months, or metastatic disease. Written informed consent was obtained from all patients.

A total of 100 patients were randomly assigned to either the ketamine group or the control group in a 1:1 ratio using a computer-generated random number table. Assignments were concealed in sealed envelopes. Both ketamine and saline were prepared in identical 50-mL syringes by a nurse who was not involved in the study to ensure the surgeons, attending anesthesiologists, and nurses involved in patient management were blinded to group assignment. Ketamine (Lake Forest, IL, USA) was diluted to a total of 50 mL (10

$mg \cdot mL^{-1}$) with saline solution. The ketamine group received a loading dose of 0.25 $mg \cdot kg^{-1}$ ketamine five minutes before skin incision, followed by a continuous infusion at 0.05 $mg \cdot kg^{-1} \cdot hr^{-1}$ until the end of surgery. These doses were determined to be within a safe dose range based on previous clinical studies of ketamine reporting that they caused no untoward psychomimetic side effects.[15,16] The control group received an equivalent volume of 0.9% saline for the same duration. Group assignment was not revealed until patients were discharged from the hospital.

Anesthetic management

In the operating room, routine monitoring included electrocardiography, pulse ox-imetry, and blood pressure (which was measured every five minutes). All patients underwent operation under general anesthesia without concomitant neuraxial blockade. Anesthesia was induced by a bolus of propofol (1.5–2 $mg \cdot kg^{-1}$) and remifentanil (1 $\mu g \cdot kg^{-1}$). Rocuronium (0.6 $mg \cdot kg^{-1}$) was used to facilitate tracheal intuba-tion. Anesthesia was maintained with 4–7 volume% desflurane and an intravenous infusion of remifentanil (0.05–0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to maintain the bispectral index within a range of 40–60 and the mean arterial pressure within 20% of the pre-induction value. Body temperature was maintained at 36.5 $\pm$ 0.5°C, and hemoglobin was maintained at $\geq$ 8 $g \cdot dL^{-1}$ with the transfusion of allogeneic packed red blood cells (RBCs) as necessary. Approximately 15 min before the end of surgery, 50 μg fentanyl for postoperative analgesia and 0.3 mg ramosetron for postoperative nausea and vomiting prophylaxis were administered. All anesthetics were discontinued at surgery completion, and 1 mg neostigmine with 0.2 mg glycopyrrolate was administered to reverse possible residual neuromuscular blockade. The endotracheal tube was removed when the patients regained consciousness and were able to breathe spontaneously. Drugs possessing anti-inflammatory effects such as dexamethasone and lidocaine were not administered in the perioperative period.

For postoperative analgesia, intravenous patient-controlled analgesia (IV-PCA) (fentanyl 15 $\mu g \cdot kg^{-1}$ and ramosetron 0.3 mg in 0.9% normal saline, with a total volume of 100 mL at the following settings: basal rate, 2 $mL \cdot hr^{-1}$; bolus, 0.5 mL; and lockout time, 15 min) was provided during the first 48 hr after surgery in both groups. In the postanesthesia care unit, intravenous oxycodone 0.1 $mg \cdot kg^{-1}$ was available as an additional analgesic for patients with an 11-point numerical pain rating scale score of 4 or greater. In the postoperative ward, both groups received tramadol (50 mg) or pethidine (25 mg) intravenously as a rescue analgesic. An investigator blinded to group assignment evaluated any psychotomimetic side effects (hallucinations or nightmares) at one, 24, and 48 hr postoperatively.

Outcome measures and other assessed variables

The primary outcome measure was NK cell activity, which was measured preoperatively and at one, 24, and 48 hr postoperatively. Natural killer cell activity was analyzed using the NK Vue kit (ATGen, Sungnam, Korea). For each patient, 1 mL whole blood was drawn from the arterial line into a NK Vue tube, which contains Promoca (a cytokine that stimulates NK cell activity) and RPMI 1640 media, and incubated at 37°C for 24 hr. The stimulatory cytokine and duration of incubation preferentially causes NK cells to secrete interferon-γ predominantly rather than other immune cells. Therefore, the level of interferon-γ in the supernatant was measured (in duplicate, and then averaged) by the NK Vue ELISA as an indicator of NK cell activity. The absolute value of NK cell activity and the proportion of patients with NK cell activity < 100 $pg \cdot mL^{-1}$ interferon-γ, representing severe immunocompromised status,[17] were evaluated at each time point.

Secondary outcomes measures included proinflammatory cytokines (interleukin-6 [IL-6] and tumour necrosis factor-α [TNF-α]) in serum using commercial ELISA kits (D6050 and HSTA00E; R&D Systems, MN, USA), and the neutrophil-lymphocyte ratio. These outcomes were measured preoperatively and again at one, 24, and 48 hr postoperatively. In addition, the C-reactive protein (CRP) level was measured preoperatively and on one, three, and five days postoperatively. The amount of intraoperative fentanyl use was calculated based on the duration and infusion rate ($\mu g \cdot kg^{-1} \cdot hr^{-1}$). Pain scores using an 11-point numerical rating scale (0 = no pain; 10 = worst pain), fentanyl dosage administered via IV-PCA, as well as the number of patients requiring additional opioid analgesics and the amount administered (morphine equivalent dose, mg) to those patients were assessed at one, 24, and 48 hr postoperatively. Carcinoembryonic antigen and cancer recurrence or metastasis (evaluated with computed tomography) was assessed every six months for two years after surgery.

Statistical analysis

The mean (standard deviation) reduction of NK cell activity at 1 day after surgery compared with baseline was 83.1 (25.2)%.[5] We aimed for a study with a 90% probability (ß = 0.1) of detecting a 20% relative decrease in the reduction of NK cell activity at a significance level (α) of 0.05. The calculated minimum sample size was 48

patients in each group. Assuming a 5% dropout rate, the final sample size was increased to 50 patients per group.

After performing Lilliefors test corrected Kolmogorov–Smirnov test for normality of distribution, continuous variables were analyzed using the Student's *t* test or Mann–Whitney U-test and expressed as means with 95% confidence interval or medians [interquartile range]. Dichotomous variables were compared using the Chi square or Fisher's exact tests and expressed as absolute numbers and percentages (%). Serially measured variables, such as NK cell activity, proinflammatory cytokines, and carcinoembryonic antigen, were log-transformed for normality of distribution. These data were analyzed using a linear mixed model with patient indicator as a random effect and group, time, and group-by-time as fixed effects. When variables with repeated measures showed significant differences between groups, *post hoc* analyses with Bonferroni correction were performed to adjust for multiple comparisons. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 25.0, IBM Corp., Armonk, NY, USA). Differences were considered significant at a $P < 0.05$.

Results

All the 100 patients completed the study without any complications (Figure). Patient characteristics and operation details, including the lowest temperature, were comparable between the two groups (Table 1). One patient in the ketamine group received a packed RBC transfusion, and no patient received other allogeneic blood components.

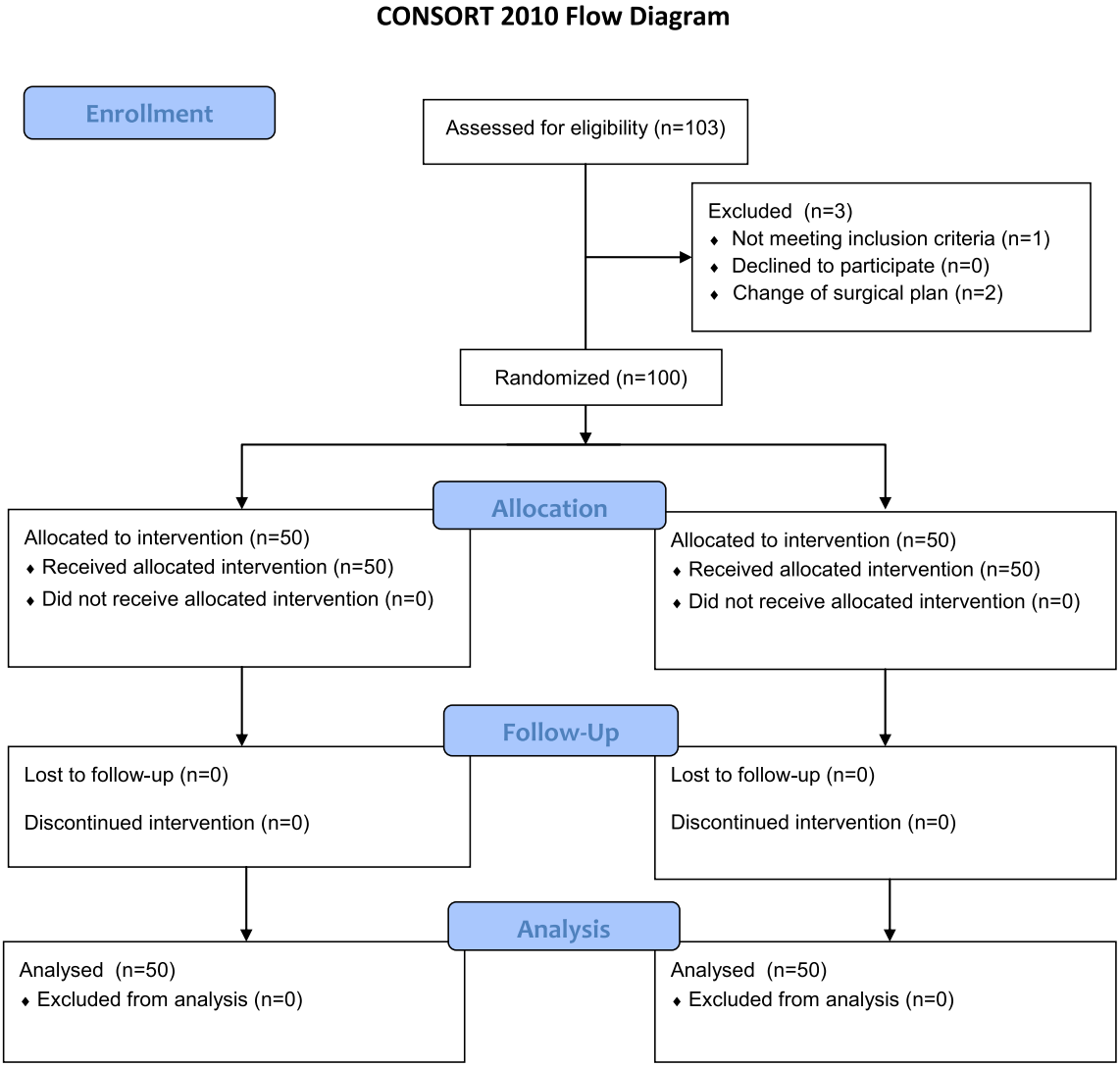

Figure Consort diagram

Table 1 Patient characteristics and operation details

Variables	Control group (n = 50)	Ketamine group (n = 50)
Age (yr)	61.0 (58.9 to 63.0)	57.2 (53.9 to 60.4)
Sex (male:female)	30:20	27:23
Body mass index ($kg \cdot m^{-2}$)	24.1 (23.2 to 25.0)	25.0 (23.0 to 26.9)
Diabetes mellitus	11 (22%)	8 (16%)
ASA physical status I/II/III	27/20/3	26/21/3
Cancer type		
Colon	37 (74%)	32 (64%)
Rectal	13 (26%)	18 (36%)
Operation		
Right-sided hemicolectomy	15 (30%)	14 (28%)
Transverse colonic resection	1 (2%)	3 (6%)
Left-sided hemicolectomy	4 (8%)	2 (4%)
Sigmoid resection	19 (38%)	17 (34%)
Low anterior resection	11 (22%)	14 (28%)
Cancer stage I/II/III/IV	10/18/ 22/0	16/16/18/0
Preoperative neoadjuvant therapy	2 (4%)	2 (4%)
Duration of operation (min)	196 (178 to 214)	199 (181 to 217)
Duration of anesthesia (min)	246 (226 to 265)	243 (223 to 263)
Intraoperative remifentanil ($\mu g \cdot kg^{-1} \cdot hr^{-1}$)*	3.6 (3.2 to 3.9)	3.0 (2.7 to 3.2)
Lowest temperature during surgery (°C)	36.0 (35.9 to 36.1)	36.1 (36.0 to 36.2)
Bleeding (mL)	20 [10–50]	20 [0–50]

Values are mean (95% confidence interval), number (%), or median [interquartile range]

ASA = American Society of Anesthesiologists

*Intraoperative remifentanil infusion rate was lower in the ketamine group than in the control group (P = 0.01), whereas other variables were not different between groups

No patient exhibited psychotomimetic side effects related to ketamine.

Natural killer cell cytotoxicity

The baseline NK cell activity was comparable between the two groups (P = 0.65), and NK cell activity decreased significantly compared with baseline in both groups after surgery. The change of NK cell activity over time was not different between the groups in the linear mixed model analysis (P = 0.47; Table 2). The proportion of patients with abnormal NK cell activity (< 100 $pg \cdot mL^{-1}$ interferon-γ) was not different between the groups preoperatively, or at one, 24, and 48 hr postoperatively (all $P > 0.05$ after Bonferroni correction.

Inflammatory responses

Perioperative inflammatory responses were comparable between the ketamine and control groups. Serum IL-6 levels before surgery were similar between the two groups (P = 0.24). At one, 24, and 48 hr after surgery, IL-6 levels were significantly higher than the baseline values in both groups (all $P < 0.05$), while the change of IL-6 was not different between groups (P = 0.27). In both groups, serum TNF-α level was greater at 48 hr after surgery than the corresponding baseline values (all $P < 0.05$). The change of TNF-α was not different between groups (P = 0.83). Similarly, the neutrophil-lymphocyte ratio and CRP levels increased after surgery in both groups compared with baseline values, whereas the changes over time were similar between groups (Table 3).

Pain scores and analgesic requirement

Pain scores assessed at one, 24, and 48 hr after surgery were similar between the two groups. Intraoperative remifentanil infusion rate was higher in the control group compared with the ketamine group (3.6 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ *vs* 3.0 $\mu g \cdot kg^{-1} \cdot hr^{-1}$, P = 0.01). Postoperative fentanyl dosage administered via IV-PCA, additional opioid requirements, and non-steroidal anti-inflammatory drug use (2 *vs* 5, P = 0.24) were similar between the control and ketamine groups (Table 4).

Carcinoembryonic antigen and prognosis

The change of carcinoembryonic antigen levels was comparable between the two groups (P = 0.97; Table 5). Among 46 patients considered for adjuvant chemotherapy (25 patients in the control group and 21 patients in the ketamine group), 23 patients (92%) in the control group and 21 patients (100%) in the ketamine group received adjuvant therapy (P = 0.19), all within eight weeks postoperatively. Two eligible patients in the control group did not receive adjuvant therapy, as one patient had anastomosis site leakage and underwent diverting loop ileostomy, while the other refused to receive chemotherapy (Table 6).

The incidences of complications, both infectious and non-infectious, were comparable between the two groups (P = 0.54). Seven patients in the control group had complications: one wound infection, one anastomosis leak, one pneumonia, three anastomosis stenosis/obstructions, and one cerebrovascular disease. Five patients in the ketamine group had complications: two wound infections, one pneumonia, one wound bleeding, and one anastomosis stenosis/obstruction. One patient in the control group died one month after surgery because of pneumonia. Four

Table 2 Natural killer cell activity

Variable time points	Control group (n = 50)		Ketamine group (n = 50)		$P_{Group\ x\ time}$
	Log-transformed	Raw	Log-transformed	Raw	
Natural killer cell activity (pg·mL^{-1})					
Before surgery (baseline)	2.36 (2.06 to 2.66)	489.3	2.45 (2.17 to 2.73)	379.0	0.47†
1 hr after surgery	1.12 (0.94 to 1.30)*	14.5	1.16 (0.95 to 1.37)*	15.0	
24 hr after surgery	1.10 (0.85 to 1.34)*	10.9	1.21 (0.95 to 1.48)*	14.5	
48 hr after surgery	1.21 (1.00 to 1.41)*	17.6	1.48 (1.25 to 1.72)*	24.5	
% Reduction from baseline					
1 hr after surgery	52.4 (45.1 to 59.6)	96.0	53.2 (45.3 to 61.2)	96.1	0.79†
24 hr after surgery	49.9 (40.6 to 59.2)	96.8	51.1 (41.6 to 60.7)	97.4	
48 hr after surgery	43.6 (35.2 to 52.1)	96.0	45.5 (36.3 to 54.7)	96.0	

Values are mean (95% confidence interval)

*$P < 0.05$ compared with before surgery; †Analyzed using linear mixed model with log-transformed data for normality of distribution

Table 3 Inflammatory responses

Variable/time points	Control group (n = 50)		Ketamine group (n = 50)		$P_{Group\ x\ time}$
	Log-transformed	Raw	Log-transformed	Raw	
Interleukin-6 (pg·mL^{-1})					
Before surgery	-0.1 (-0.3 to 0.1)	0.9	0.1 (-0.1 to 0.3)	0.8	0.27†
1 hr after surgery	1.4 (1.3 to 1.6)*	31.3	1.3 (1.2 to 1.5)*	26.3	
24 hr after surgery	1.5 (1.4 to 1.6)*	37.5	1.5 (1.4 to 1.6)*	37.3	
48 hr after surgery	1.3 (1.1 to 1.4)*	20.3	1.2 (1.1 to 1.3)*	16.0	
Tumour necrosis factor-α (pg·mL^{-1})					
Before surgery	0.2 (0.2 to 0.3)	1.7	0.2 (0.1 to 0.2)	1.4	0.83†
1 hr after surgery	0.2 (0.2 to 0.3)	1.6	0.2 (0.1 to 0.2)	1.5	
24 hr after surgery	0.3 (0.2 to 0.3)	1.9	0.2 (0.1 to 0.3)	1.8	
48 hr after surgery	0.4 (0.3 to 0.4)*	2.2	0.3 (0.3 to 0.4)*	2.1	
Neutrophil-lymphocyte ratio					
Before surgery	0.3 (0.2 to 0.3)	2.0	0.3 (0.2 to 0.4)	2.1	0.69†
1 hr after surgery	0.8 (0.7 to 0.9)*	6.3	0.7 (0.6 to 0.8)*	6.3	
24 hr after surgery	0.7 (0.7 to 0.8)*	5.5	0.7 (0.7 to 0.8)*	5.2	
48 hr after surgery	0.7 (0.7 to 0.8)*	5.4	0.7 (0.6 to 0.8)*	4.9	
C-reactive protein (mg·L^{-1})					
Before surgery	0.32 (0.08 to 0.56)	2.20	0.05 (-0.20 to 0.30)	1.70	0.99†
1 day after surgery	1.49 (1.42 to 1.56)*	30.90	1.56 (1.49 to 1.62)*	40.85	
3 days after surgery	1.76 (1.69 to 1.84)*	61.60	1.81 (1.73 to 1.90)*	71.10	
5 days after surgery	1.48 (1.36 to 1.60)*	27.85	1.50 (1.40 to 1.61)*	34.50	

Values are mean (95% confidence interval)

*$P < 0.05$ compared with before surgery; †Analyzed using linear mixed model with log-transformed data for normality of distribution

patients (8.5%) in the control group and five patients (11.6%) in the ketamine group had cancer recurrence and/or metastasis during the two-year follow-up period after surgery (P = 0.62).

Discussion

In this prospective randomized-controlled trial, intraoperative subanesthetic low-dose ketamine added as an adjunct to desflurane anesthesia did not convey any significant beneficial effect on postoperative NK cell

Table 4 Pain scores and analgesic requirements

Variable/time points	Control group (n = 50)	Ketamine group (n = 50)	P value
Pain score (resting/activity)			
1 hr after surgery	3 [3–4]/ 4 [3–5]	3 [3–5]/ 5 [3–5]	0.86/0.62
24 hr after surgery	3 [2–5]/ 5 [3–7]	3 [2–4]/ 5 [4–7]	0.80/0.78
48 hr after surgery	2 [0–3]/ 4 [3–5]	3 [2–3]/ 5 [3–6]	0.14/0.51
Fentanyl administered via intravenous patient-controlled analgesia (μg)			
0–24 h after surgery	548 (508 to 587)	559 (511 to 606)	0.73
24–48 h after surgery	389 (342 to 435)	355 (308 to 402)	0.30
Additional opioid analgesics requirement (morphine equivalent dose, mg)			
0–1 hr after surgery (n)	3.3 [3.0–5.0] (26)	4.0 [3.0–4.0] (29)	0.66 (0.55)
1–24 hr after surgery (n)	6.6 [3.3–9.9] (30)	6.6 [3.3–9.9] (39)	0.97 (0.05)
24–48 hr after surgery (n)	6.6 [3.7–10.0] (24)	6.6 [3.3–9.6] (28)	0.69 (0.42)

Values are median [interquartile range], mean (95% confidence interval) or number

Pain score, a numerical pain intensity scale (0 = no pain, 10 = the worst pain)

Table 5 Carcinoembryonic antigen

Time points	Control group (n = 50)		Ketamine group (n = 50)		P $^{Group\ x\ time}$
	Log-transformed	Raw	Log-transformed	Raw	
Before surgery ($ng \cdot mL^{-1}$)	0.38 (0.24 to 0.52)	2.00	0.51 (0.36 to 0.66)	2.35	0.97†
6 months after surgery ($ng \cdot mL^{-1}$)	0.27 (0.15 to 0.40)	1.74	0.31 (0.23 to 0.39)	2.21	
1 year after surgery ($ng \cdot mL^{-1}$)	0.18 (0.09 to 0.28)*	1.41	0.29 (0.22 to 0.36)*	2.03	
2 years after surgery ($ng \cdot mL^{-1}$)	0.25 (0.15 to 0.35)	1.60	0.38 (0.30 to 0.46)	2.45	

Values are mean (95% confidence interval)

*$P < 0.05$ compared with before surgery; †Linear mixed model analysis with log-transformed data for normality of distribution

Table 6 Postoperative adjuvant therapy

	Control group (n = 50)	Ketamine group (n = 50)	P value
Patients eligible for adjuvant therapy	25 (50%)	21 (42%)	0.42
Patients receiving adjuvant therapy	23/25 (92%)	21/21 (100%)	0.19
Patients not receiving adjuvant therapy	2/25 (8%)	0	

Values are number (%)

activity and proinflammatory cytokine levels in patients undergoing colorectal cancer surgery.

The perioperative period is regarded as critical for cancer dissemination and metastasis because it can result in impaired anti-cancer immune surveillance, excessively increased growth factors during the wound healing process, and the release of cancer cells into the circulation during surgical manipulation.[18] Suppressed cell-mediated immunity (mainly NK cells and T lymphocytes) and excessive proinflammatory responses are pivotal characteristics of perioperative cytokine cascade activation.[4,10] The balance between perioperative factors promoting cancer survival and growth and the host's antitumour defenses can determine whether the residual tumour cells lead to clinical deterioration.[1] In this respect, because anesthetics and opioid analgesics interact with the immune system, their effect on facilitating or hindering tumour growth and metastasis has surfaced as a critical issue.[9]

Ketamine interacts with many receptors and ionic channels, with its main action occurring via NMDA receptor antagonism. The NMDA-activated glutamate receptors are expressed in lymphocytes and have excitotoxic effects related to lymphocyte immunocompetence.[14] The NMDA receptor activation increases intracellular calcium and reactive oxygen species levels in both NK and T cells. Additionally, NMDA receptors are expressed in cancer cells,[19] and their

inhibition could diminish the growth of cancer cells by decreasing intracellular Ca^{2+} levels that are of vital importance in tumour progression.[20] Ketamine has been shown to have antitumour effects by blocking the NMDA receptor in various subsets of cancer cells[21,22] including colon adenocarcinoma cells.[23] Ketamine attenuated vascular endothelial growth factor expression and cell migration ability[23] and decreased aerobic glycolysis, the main energy source of cancer.[24] Furthermore, ketamine showed protective effects against cellular immune impairment and cancer metastasis induced by surgical stress.[25] Nevertheless, contradictory results have been reported when using anesthetic or higher doses.[11]

Because no previous clinical studies have found evidence of this protective effect, we investigated the effect of a subanesthetic dose of ketamine on NK cell activity and cytokine activities in patients undergoing colorectal cancer surgery. In these patients, NK cells act as the main defense against tumour growth and metastasis. The NK cells are a critical part of innate immunity[3] and have direct cellular cytotoxicity against tumours.[2] Patients with low NK cell activity had a ten-fold higher risk of colorectal cancer than patients with high NK cell activity.[26] Both impaired NK cell activity and decreased intratumoral NK cell infiltration were associated with increased recurrence and mortality in patients with colorectal cancer.[6,7] In an animal model mimicking surgical stress in humans, ketamine increased NK cell activity under surgical conditions and decreased the number of lung metastases induced by MADB-106 cells.[25] Nevertheless, in the present study, intraoperative use of low-dose ketamine did not attenuate the decrease in NK cell activity after colorectal cancer surgery. Similar results were reported in studies for non-cancer surgery patients wherein only a smaller amount of preoperative ketamine bolus was administered.[12,27]

Proinflammatory cytokines such as IL-6 and TNF-α promote proliferation and survival of cancer cells while suppressing effector cells of antitumour immunity including NK cells, $CD4^+$ T helper 1-type cells, and $CD8^+$ cytotoxic T cells.[10] Ketamine produced an anti-inflammatory effect by inhibiting excessive systemic inflammation without interfering with local healing process *in vivo*.[28] Furthermore, its anti-inflammatory effect was shown in several clinical trials[12,29] and a meta-analysis.[16] By contrast, an anti-inflammatory effect of ketamine was not observed in this study during which conditions associated with inflammatory reactions were similar between the groups. This result may be partly attributable to relatively less postoperative inflammatory activation in the present study than in previous studies due to the nature of laparoscopic surgery. Previous studies in colorectal surgery suggested that open laparotomy resulted in two-fold higher concentrations in IL-6 and CRP compared with in laparoscopic procedures.[30,31]

Poorly controlled pain can increase the risk of cancer metastasis by suppressing the lymphocyte response and NK cell activity and increasing proinflammatory cytokines.[32] Ketamine is known to possess analgesic effects and the opioid-sparing effects of perioperative ketamine are well established.[33] Ketamine was also used for refractory cancer pain,[34] although the limited literature is not conclusive about the beneficial effect of ketamine on cancer pain.[35] In the present study, the intraoperative remifentanil infusion rate was higher in the control group than the ketamine group. Co-administration of ketamine might have reduced the remifentanil requirement in the ketamine group. Nevertheless, ketamine had no favourable effects on postoperative pain and opioid consumption. The relatively low pain intensity and less additional opioid requirement attributable to the use of PCA with background infusion (a standard PCA method in our institution) in both groups, in contrast to the use of PCA without background infusion in previous studies showing the opioid-sparing effect of ketamine,[36,37] seems to be a possible explanation for the negative result.

Carcinoembryonic antigen and computed tomography imaging have been verified as the effective follow-up modes with significant potential to detect curatively, treatable metastatic recurrence in patients with colorectal cancer.[38] In the current study, the change of carcinoembryonic antigen levels and the prevalence of recurrence or metastasis during two years after surgery were not different in the ketamine and control groups; however, the sample size was not calculated to address these secondary outcome measures with sufficient statistical power.

Limitations

Limitations of this study include the possible complex influence of various anesthetic regimens. Both volatile anesthetics and opioids have been shown to suppress NK cell activity, although clinical evidence to date is inconclusive. Evidence is even more scarce regarding the immunomodulatory influence of desflurane, which seems to be less than that of sevoflurane or isoflurane.[39] Likewise, the influence of higher intraoperative remifentanil requirement on NK cell activity and inflammatory response in the control group than in the ketamine group cannot be excluded, although remifentanil in clinically relevant doses does not impair NK cell function.[40] Second, the doses used in *in vitro* or *in vivo* experiments showing a protective effect of ketamine on suppression of NK cell activity were much higher than our chosen dose,[25] and the immunomodulatory effect of ketamine was reported to be

dose-dependent,[28] which might have influenced our results. Lack of effect in the present study might have reflected an inadequate dose of ketamine. Nevertheless, anesthetic or higher doses inevitably accompany psychotomimetic side effects and sympathetic stimulation that may even result in adverse immunomodulation.[41] Thus, the dose used in the present study was carefully determined according to previous studies showing low-dose ketamine's anti-inflammatory effect[16] while minimizing the chance of introducing side effects.

Conclusions

When added as an adjunct to desflurane anesthesia, subanesthetic low-dose ketamine did not exert beneficial immunomodulatory influences in terms of perioperative NK activity and inflammatory cytokines in patients undergoing curative laparoscopic colorectal cancer surgery.

Author contributions *Jin Sun Cho* contributed to all aspects of this manuscript, including study conception and design; acquisition, analysis, and interpretation of data; and drafting the article. *Na Young Kim* contributed to conception and design of the study and acquisition of data. *Jae-Kwang Shim* contributed to interpretation of data and drafting and revising the article. *Sugeun Lee* and *Ji Hae Jun* contributed to acquisition and analysis of data. *Young-Lan Kwak* contributed to all aspects of this manuscript, including study conception and design; acquisition, analysis, and interpretation of data; and drafting and revising the article.

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