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ORIGINAL ARTICLE

Comparative effects of flurbiprofen and fentanyl on natural killer cell cytotoxicity, lymphocyte subsets and cytokine concentrations in post-surgical intensive care unit patients: prospective, randomized study

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Abstract
Purpose The purpose of this study was to compare the effect of the long-term administration of flurbiprofen and fentanyl in the intensive care unit on natural killer cell cytotoxicity (NKCC), lymphocyte subsets and cytokine levels.
Methods In this prospective study, patients scheduled for at least 48 h sedation after neck surgery were randomly assigned to two groups called group N and group F. Group N patients were sedated with propofol and flurbiprofen after surgery ($n = 12$), while group F patients were sedated with propofol and fentanyl ($n = 13$). The NKCC, lymphocyte subsets, and plasma levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 were measured before and at the end of surgery, on postoperative day (POD) 1 and POD2.
Results The NKCC was significantly higher on POD1 in group N than in group F (14.5 ± 11.2 versus 6.3 ± 4.1 %, $p < 0.05$), the difference between the groups disappearing on POD2. Lymphocyte subsets and plasma levels of cytokines were not significantly different between the two groups during the study period.
Conclusions Transient suppressive effects on NKCC were observed in the fentanyl group as compared to the flurbiprofen group. This suggests that when choosing postoperative analgesics, physicians should bear in mind the potential immunosuppressive effects of these agents in patients requiring prolonged sedation in the intensive care unit.

Keywords Natural killer cell cytotoxicity · Fentanyl · Flurbiprofen · Long-term sedation

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Introduction

There have been many reports showing that immunosuppression associated with surgical stress occurs during the perioperative period [1–3]. In addition, recent research has shown that anesthetic and analgesic drugs that are used during surgery and in the intensive care unit (ICU) possibly directly influence immunocompetent cells [4].

Appropriate sedation and analgesia for the ventilated patient in the ICU are important therapeutic techniques that affect recovery from surgical stress in the postoperative period. Currently, of the many sedative and/or analgesic agents used for ventilated patients in the ICU, opiates are one of the most commonly used analgesic agents [5], with morphine being one of the most useful of them [6]. However, there are many reports showing that morphine may exert various suppressive effects on the immune system [7, 8]. Hence, morphine may not be desirable for analgesia in immunocompromised patients. Fentanyl is another useful opiate for analgesia [9]. Previously, we found that the addition of low-dose fentanyl to propofol provides effective sedation in ICU patients after cardiac surgery [10]. Using animal studies, Shavit et al. [11] reported that fentanyl decreases natural killer cell cytotoxicity (NKCC) and increases tumor metastasis. The same group [12] also observed that the immunosuppressive effects of fentanyl were more prolonged in the high-dose fentanyl group (75–100 μg/kg) compared with those in the low-dose

fentanyl group (up to 6 μg/kg) in patients undergoing abdominal surgery.

Non-steroidal anti-inflammatory drugs (NSAID) are also commonly used analgesic agents that produce pain relief effects different from those of opiates. The NSAIDs reportedly enhance immunocompetent cells, such as macrophages, natural killer (NK) cells and T lymphoid cells [13].

There are many reports examining the effects of fentanyl on the immune system and cytokine levels in animal and clinical studies. However, the observation periods in most of these reports were relatively short (up to 24 h), with no clinical study investigating the effects on the immune system of long-term (over 24 h) administration of fentanyl postoperatively in the ICU. We speculated that long-term administration of fentanyl (more than 24 h) in postoperative patients in the ICU may have different effects on the immune system as compared to NSAIDs.

The present study was conducted to compare the effects of long-term administration of flurbiprofen (non-selective NSAID) and fentanyl on NKCC, lymphocyte subsets and concentration of cytokines in postoperative ICU patients.

Methods

Institutional and ethics committee approval was obtained for this study. After obtaining their written informed consent, 25 patients were included in this study during the period from August 2011 to October 2012. They were classified as either grade 1 or 2 according to the American Society of Anesthesiologists (ASA) grading of physical status. We selected neck cancer patients who were scheduled for planned sedation for at least 48 h or more after the operation to ensure stabilization of their neck.

Patients who had recently taken corticosteroids, those with endocrine diseases or hematological malignancy, young patients <20 years of age, dialysis patients, patients who were ASA physical status 3, 4 or 5, those with atrioventricular conduction block greater than first degree and patients with a history of drug allergy were excluded from the study. Additional exclusion criteria included history of asthma, bronchospasm, chronic obstructive pulmonary disease, coronary artery disease, heart rate <50 beats/min and systolic blood pressure <80 mmHg at the time of entry into the operation room, active liver disease (glutamine oxaloacetate transaminase or glutamine pyruvate transaminase >50 U/dL), renal dysfunction (plasma creatinine level ≥2.0 mg/dL), cerebrovascular disease and psychiatric illness.

The participants were randomly assigned to one of two methods of sedation after arrival in the ICU and the two groups were called group N (NSAID) and group F (fentanyl).

In both groups, the following anesthetic regimen during the surgery was identical: anesthesia was induced with propofol (1–2 mg/kg), with rocuronium (0.9 mg/kg) being administered after loss of consciousness. Anesthesia was maintained with sevoflurane (1.0–2.0 vol%, end-tidal concentration) in 4 L/min air/oxygen (fraction of inspired oxygen, 0.4–0.5), remifentanil (0.05–0.5 μg/kg/min) and bolus doses of fentanyl (25–100 μg). Bispectral index (version 3.0; Aspect Medical Systems, Newton, MA, USA) was measured continuously on an electroencephalographic monitor (model A-2000; Aspect Medical Systems) using a BIS Sensor strip (Aspect Medical Systems). Impedance of each electrode was maintained at <2 kΩ. Target BIS values of 45–55 were achieved by modulating sevoflurane concentrations.

After surgery, patients in both groups were transferred to the ICU and mechanically ventilated for at least 48 h after the surgery, due to the need for immobilization of the surgical site. During this period, group N patients were sedated with propofol and flurbiprofen, while group F patients were sedated with propofol and fentanyl. Clinical physicians evaluated Richmond Agitation-Sedation Scale (RASS) [14] and Behavioral Pain Scale (BPS) scores [15] at hourly intervals. We controlled the dose of propofol to keep RASS in the range of −3 to −1, and adjusted the dose of flurbiprofen and fentanyl to maintain BPS in the range of 3–5. The sedative and analgesic drug administration protocol in the ICU was as below. After ICU admission, propofol was administered at 3 mg/kg/h in both groups. After the hourly evaluation, the dosing rate of propofol was reduced by 0.5 mg/kg/h if RASS was <−3, and increased by 0.5 mg/kg/h if RASS was more than −1. In group N, flurbiprofen was commenced at 0.1 mg/kg/h. At every hourly evaluation, 0.1 mg/kg of flurbiprofen was infused and the dosing rate was increased by 0.02 mg/kg/h if BPS was more than 5. In group F, fentanyl was commenced at 1 μg/kg/h. At every hourly evaluation, 1 μg/kg of fentanyl was infused and the dosing rate was increased by 0.2 μg/kg/h if BPS was more than 5.

Arterial blood samples (15 mL) were collected before and at the end of surgery, on postoperative day (POD) 1 and POD2. From these samples, 5 mL of blood was used for analysis of NKCC and 5 mL was used for assay of lymphocyte subsets. The rest of the sample was centrifuged, and the plasma was frozen at −80 °C. Then, the plasma levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-10 were measured by enzyme-linked immunosorbent assay kits. At each point, white blood cells (WBC), C-reactive proteins (CRP) and glucose were measured as routine blood sampling.

The NKCC was measured by a standard chromium-51 (^{51}Cr) assay using radiolabeled K562 erythroleukemia cell lines as targets [16]. Fresh peripheral-blood mononuclear cells (PBMCs) were isolated by gradient centrifugation (at 1800 rpm for 20 min) of the blood sample and irrigated with phosphate buffered saline (PBS) three times. Cell populations were titrated for cytolytic activity against 5,000 ^{51}Cr-labeled K562 target cells. Finally, effector-cell suspensions were adjusted to 1×10^6 cells/mL, and an effector-to-target cell ratio of 20:1 was tested. Plates were centrifuged for 5 min at 800 rpm before incubation for 3.5 h at 37 °C in 5 % CO_2, and centrifuged again for 5 min at 1,500 rpm before collecting equal volumes of supernatant from each well. The radioactivity in the supernatant was determined with a γ counter. The NKCC was calculated as follows: [counts per minute, cpm (experimental) − cpm (control)] × 100/[cpm (maximum) − cpm (control)], where cpm (experimental) = counts after incubation of target cells with effector cells, cpm (maximum) = counts after incubation of target cells in 1 N HCl, and cpm (control) = counts after incubation of target cells in medium alone. The NKCC was measured in triplicate.

Lymphocyte subsets were analyzed by flow cytometry (FACScalibur; BD Biosciences) with fluorescein isothiocyanate-labeled monoclonal antibodies (Beckman Coulter) specific to the cell markers. The following antibodies to lymphocyte antigens were used and cell types determined [17]: cluster of differentiation (CD)3+ (T lymphocytes), CD4+ (helper T lymphocytes), CD8+ (suppressor and cytotoxic T lymphocytes), CD16+ (predominantly NK cells), and CD19+ (B lymphocytes). The percentage of each type of lymphocyte relative to the total lymphocytes was determined.

Statistical analysis

Data were analyzed at a later time by an individual who was blind to the treatment regimens. Before the start of this study protocol, we calculated sample size. Based on a previous study [16], we hypothesized that NKCC would decrease by 10 % in group F compared with that in group N. We determined that 12 members in each group were required to provide a 70 % power to detect a 30 % difference between group F and group N.

All data are expressed as means ± standard deviation (SD). Following the confirmation of equal variance among the groups by the Bartlett test, changes in mean values of hemodynamic variables, cytokine levels and NKCC (baseline and between groups) were compared using one one-way factorial measure or two-way repeated measures ANOVA. When the F value was significant, the Bonferroni method was used for multiple comparisons. Demographic data of the two groups were analyzed by the unpaired-t test. Values of $p < 0.05$ were considered statistically

Table 1 Patient characteristics and doses of drugs in the two groups

	Group N	Group F	p value
Number of patients (male/female)	12 (10/2)	13 (10/3)	
Age (years)	66.8 ± 12.9	60.4 ± 15.7	0.28
Height (cm)	164.4 ± 11.3	162.6 ± 9.7	0.67
Weight (kg)	63.9 ± 17.1	58.0 ± 12.3	0.33
Duration of surgery (min)	954 ± 238	978 ± 263	0.82
Duration of anesthesia (min)	1025 ± 232	1047 ± 258	0.82
Intraoperative blood loss	1347 ± 850	1642 ± 1211	0.49
Intraoperative blood transfusion	822 ± 1060	816 ± 1286	0.99
Intraoperative fluid balance (mL)	3622 ± 2086	4310 ± 1112	0.31
Total intraoperative dose of fentanyl (mg)	0.71 ± 0.20	0.69 ± 0.42	0.88
Total intraoperative dose of fentanyl per kg body weight (mg/kg)	0.012 ± 0.005	0.012 ± 0.007	0.98
Total intraoperative dose of remifentanil (mg)	12.9 ± 5.1	10.5 ± 5.3	0.29
Total intraoperative dose of remifentanil per kg body weight (mg/kg)	0.205 ± 0.077	0.178 ± 0.067	0.35
Total intraoperative dose of sevoflurane (mL)	380 ± 108	381 ± 113	0.99
Total ICU dose of propofol (mg)	9314 ± 4738	6745 ± 3201	0.13
Total ICU dose of propofol per kg body weight (mg/kg)	149 ± 57	114 ± 42	0.10
Total ICU dose of flurbiprofen (mg)	324 ± 144		
Total ICU dose of flurbiprofen per kg body weight (mg/kg)	5.78 ± 1.98		
Total ICU dose of fentanyl (mg)		4.00 ± 1.16	
Total ICU dose of fentanyl per kg body weight (mg/kg)		0.071 ± 0.023	

Values are expressed as mean ± SD

ICU intensive care unit, *group N* non-steroidal anti-inflammatory drug group, *group F* fentanyl group

Table 2 Time course of changes in vital signs, respiratory status and laboratory data in the two groups

	Group	Pre-Op	End-Op	POD1	POD2
MAP (mmHg)	Group N	93.7 ± 15.0	90.6 ± 16.4	75.0 ± 6.8†	81.1 ± 11.5†
	Group F	91.0 ± 19.5	87.1 ± 12.0	79.6 ± 6.4†	81.1 ± 8.6†
HR (beats/min)	Group N	74.9 ± 15.1	85.8 ± 10.8†	72.2 ± 12.7	70.7 ± 14.2
	Group F	75.2 ± 12.8	89.0 ± 14.0†	76.2 ± 10.7	70.2 ± 11.9
BT (°C)	Group N	36.4 ± 0.51	36.9 ± 0.49†	36.8 ± 0.41*,†	37.2 ± 0.67*,†
	Group F	36.4 ± 0.45	37.0 ± 0.44†	37.1 ± 0.49†	37.5 ± 0.57†
P/F ratio (mmHg)	Group N	427.3 ± 96.3	370.4 ± 97.0†	397.3 ± 58.2†	382.8 ± 95.1†
	Group F	464.3 ± 122.6	407.0 ± 91.2†	406.8 ± 79.5†	383.4 ± 88.5†
PEEP (cmH_2O)	Group N	None	5.0 ± 0.0	4.7 ± 0.8	3.2 ± 2.4
	Group F	None	5.0 ± 0.0	4.3 ± 1.5	2.7 ± 2.6
Glucose (mg/dL)	Group N	114.7 ± 14.0	154.0 ± 35.2†	133.5 ± 30.8†	127.5 ± 15.7
	Group F	122.5 ± 30.0	161.2 ± 42.0†	144.3 ± 33.0†	133.0 ± 27.9
WBC ($\times 10^3/\mu L$)	Group N	5.63 ± 1.06	11.0 ± 3.21†	12.03 ± 2.67†	10.78 ± 2.37†
	Group F	5.50 ± 0.98	10.69 ± 3.21†	12.21 ± 3.43†	10.96 ± 3.26†
CRP (mg/dL)	Group N	0.07 ± 0.07	5.62 ± 3.89†	11.42 ± 6.94†	8.48 ± 6.28†
	Group F	0.12 ± 0.21	5.77 ± 5.45†	13.37 ± 6.86†	9.72 ± 3.92†

Values are expressed as mean ± SD

Group N non-steroidal anti-inflammatory drug group, *group F* fentanyl group, *MAP* mean arterial pressure, *HR* heart rate, *BT* body temperature, *P/F ratio* ratio of partial pressure of oxygen/fraction of inspired oxygen, *PEEP* positive end-expiratory pressure, *WBC* white blood cell, *CRP* C-reactive protein, *Pre-Op* before operation, *End-Op* end of operation, *POD* post operative day

* $p < 0.05$ versus group F

† $p < 0.05$ versus the level before operation

significant. All calculations were performed on a Macintosh computer (Apple Co.) using StatView 5.0 software (Abacus Concepts, Berkeley, CA, USA).

Results

Patient characteristics, duration of surgery and anesthesia, amount of blood lost, amount of blood transfused, fluid balance during the operation, total dose of fentanyl, remifentanil and sevoflurane during the operation, and total dose of propofol in the ICU were not significantly different between the two groups (Table 1). Total dose of flurbiprofen in the ICU was 324 ± 144 mg (5.78 ± 1.98 mg/kg). Total dose of fentanyl in the ICU was 4.00 ± 1.16 mg (0.071 ± 0.023 mg/kg).

Changes in vital signs, respiratory status and laboratory data are shown in Table 2. Body temperature was significantly lower on POD1 and POD2 in group N as compared to group F ($p < 0.05$). There were no significant differences in any other values between the groups. In both groups, the following postoperative values differed significantly in comparison to their respective levels before the operation (Table 2). Mean arterial pressure decreased significantly on POD1 and POD2 in both groups ($p < 0.05$). Heart rate increased significantly at the end of operation in

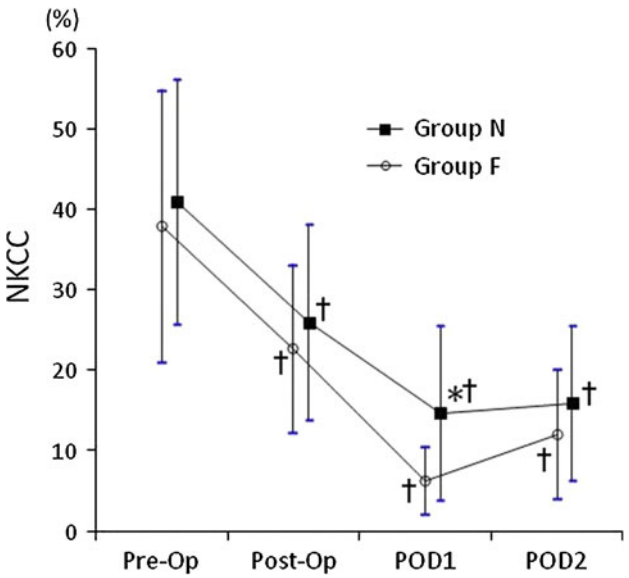

Fig. 1 Time course of changes in natural killer cell cytotoxicity (NKCC) in the two groups. On POD1, NKCC was significantly higher in group N than in group F ($p < 0.05$), the value decreasing significantly at the end of the operation and on POD1 and POD2 in each group compared to the corresponding level before operation ($p < 0.05$). Values are expressed as mean ± SD. *Group N* non-steroidal anti-inflammatory drug group, *group F* fentanyl group, *NKCC* natural killer cell cytotoxicity, *Pre-Op* before operation, *End-Op* end of operation, *POD* postoperative day. *$p < 0.05$ versus group F; †$p < 0.05$ versus the level before operation

Table 3 Time course of changes in lymphocyte subsets in the two groups

	Group	Pre-Op	End-Op	POD1	POD2
CD3+ (%)	Group N	63.4 ± 14.8	69.4 ± 9.7	61.7 ± 10.0	67.8 ± 9.8
	Group F	67.3 ± 11.9	67.9 ± 16.6	56.5 ± 19.0†	64.7 ± 15.1
CD4+ (%)	Group N	36.9 ± 10.3	42.1 ± 10.5	36.4 ± 11.1	40.3 ± 11.2
	Group F	41.3 ± 11.8	43.6 ± 15.0	32.6 ± 12.3†	40.5 ± 10.8
CD8+ (%)	Group N	37.6 ± 13.1	33.7 ± 12.6	34.2 ± 12.4	34.2 ± 14.0
	Group F	34.7 ± 7.6	33.0 ± 9.6	31.8 ± 6.2	32.2 ± 7.6
CD16+ (%)	Group N	28.5 ± 11.1	22.5 ± 9.2	22.9 ± 11.3	22.6 ± 10.5
	Group F	25.1 ± 13.8	21.9 ± 11.2	23.3 ± 11.8	20.9 ± 11.1
CD19+ (%)	Group N	5.8 ± 2.8	8.3 ± 5.3	12.7 ± 12.1†	11.8 ± 8.7†
	Group F	7.1 ± 3.8	8.7 ± 5.1	16.1 ± 12.9†	14.4 ± 10.2†

Values are expressed as mean ± SD

Group N non-steroidal anti-inflammatory drug group, *group F* fentanyl group, *CD* cluster of differentiation, *Pre-Op* before operation, *End-Op* end of operation, *POD* post operative day

† $p < 0.05$ versus Pre-Op

Table 4 Time course of changes in plasma concentrations of cytokines in the two groups

	Group	Pre-Op	End-Op	POD1	POD2
TNF-α (pg/mL)	Group N	0.88 ± 0.45	1.74 ± 1.14†	0.80 ± 0.29	1.14 ± 0.64
	Group F	0.96 ± 0.54	2.02 ± 1.61†	1.04 ± 0.61	1.06 ± 0.43
IL-1β (pg/mL)	Group N	0.14 ± 0.04	0.66 ± 0.69†	0.18 ± 0.12	0.22 ± 0.15
	Group F	0.15 ± 0.08	0.81 ± 0.78†	0.26 ± 0.23	0.23 ± 0.18
IL-6 (pg/mL)	Group N	3.9 ± 3.2	478.7 ± 550.9†	44.3 ± 47.6	60.4 ± 65.5
	Group F	3.1 ± 3.7	506.9 ± 397.8†	77.2 ± 70.2	60.3 ± 43.7
IL-10 (pg/mL)	Group N	0.65 ± 0.37	4.03 ± 4.75†	1.26 ± 1.11	1.24 ± 1.05
	Group F	0.51 ± 0.03	4.30 ± 6.36†	1.30 ± 1.17	1.61 ± 1.72

Values are expressed as mean ± SD

Group N non-steroidal anti-inflammatory drug group, *group F* fentanyl group, *TNF* tumor necrosis factor, *IL* interleukin, *Pre-Op* before operation, *End-Op* end of operation, *POD* post operative day

† $p < 0.05$ versus Pre-Op

both groups ($p < 0.05$). Body temperature, WBC count and C-reactive proteins all increased significantly at the end of surgery and on POD1 and POD2 in both groups ($p < 0.05$). The ratio of the partial pressure of oxygen/fraction of inspired oxygen decreased significantly at the end of the operation and on POD1 and POD2 in both groups ($p < 0.05$). Blood glucose increased significantly at the end of the operation and on POD1 in both groups ($p < 0.05$).

On POD1, NKCC was significantly higher in group N than in group F ($p < 0.05$) (Fig. 1). In both groups, NKCC decreased significantly at the end of the operation and on POD1 and POD2 compared to the level before surgery ($p < 0.05$).

In both groups, the following postoperative lymphocyte subsets differed significantly in comparison to their levels before the operation (Table 3). Both CD3+ and CD4+ lymphocytes decreased significantly on POD1 in group F. CD19+ lymphocytes increased significantly on POD1 and POD2 in both groups. There were no significant inter-group differences in any lymphocyte subsets.

In both groups, the plasma concentrations of TNF-α, IL-1β, IL-6 and IL-10 were all significantly increased at the end of the operation in comparison to the level before surgery ($p < 0.05$: Table 4).

Serum creatinine levels, blood urea nitrogen and estimated glomerular filtration rates were within the standard range during the study in both groups (data not shown). There were no respiratory infections, urinary tract infections or sepsis within 28 days after the operation in both groups.

Discussion

The present study shows that: (1) NKCC in group F was lower than that in group N on POD1, and the decreased NKCC in group F returned to the same value as group N on POD2, (2) no differential effects of fentanyl or flurbiprofen on cytokine and lymphocyte levels were found between the two analgesic agents.

Many diverse sedative and analgesic agents have been used in the ICU. According to a previous study [18], propofol is one of the most commonly used sedative agents, while opiates, such as morphine or fentanyl, are commonly

used for analgesia. The combination of sedative and analgesic agents provides effective therapy in ventilated patients in the ICU. Previously, we examined the effects of propofol alone and propofol plus fentanyl on hemodynamic variables in patients in the ICU after cardiac surgery, and found that the combination of propofol plus fentanyl was superior to propofol alone in terms of maintaining an adequate level of sedation together with stable systemic hemodynamics [10].

Many reports have examined the effects of analgesic drugs on the immune system [19, 20]. Morphine, which is the longest-studied opiate, has been reported to suppress many kinds of immunocompetent cells. Yeager et al. [8] administered low dose (0.025 mg/kg loading dose followed by a 0.015 mg/kg/h infusion) or high dose (0.05 mg/kg loading dose followed by a 0.03 mg/kg/h infusion) morphine to healthy volunteers for 24 h and measured NKCC the week before morphine exposure (baseline), 2 h after the initiation of intravenous morphine (2 h), at the end of the morphine infusion (24 h), 24 h after termination of the morphine infusion (48 h), and 7–10 days after termination of the morphine infusion (8 days). Depression of NKCC was observed at 2 and 24 h in both groups. Recovery of NKCC was apparent by the 48 h measurement period in the low dose group, but remained significantly depressed in the high dose group. Lysle et al. [21] administered morphine (0, 5.0, 10.0, 15.0 or 25.0 mg/kg) to rats for the purpose of determining whether morphine's immunomodulatory effects are dose-dependent. As a result, morphine induced a dose-dependent suppression of splenic lymphocyte function as measured by mitogen-induced proliferation, NK cytotoxicity, IL-2 production and interferon production, indicating that morphine exerts dose-dependent suppression of the mitogen-induced proliferative response of blood lymphocytes, while also decreasing the absolute number of blood leukocytes.

In contrast to the possible adverse effects of morphine on the immune system, controversial results exist regarding the effects of fentanyl on the immune system. Beilin et al. [12] examined the comparative effects of large doses (75–100 μg/kg) versus small doses (5 μg/kg) of fentanyl on NKCC during anesthesia, and showed that large doses of fentanyl during anesthesia caused prolonged suppression of NKCC for at least 48 h after the surgery. Subsequently, a report from the same group [22] examined the influence of three different doses of fentanyl on the immune system and showed that fentanyl suppresses the immune system in a dose-dependent manner. In contrast to the reports showing fentanyl-induced suppressive effects on NKCC, some researchers reported no immunosuppressive effects of fentanyl on NKCC in some in vivo and in vitro studies [23, 24]. Yeager et al. [24] administered fentanyl to healthy human subjects as an initial intravenous dose of 3 μg/kg, followed by a continuous infusion at the rate of 1.2 μg/kg/h for 2 h. The NKCC was tested at baseline, at the end of the fentanyl infusion, and at 1 and 24 h after the fentanyl infusion. Fentanyl produced a significant increase in NKCC at the end of the infusion. In the same study, CD16+ lymphocytes, which are predominantly NK cells, increased significantly in peripheral blood at the end of the fentanyl infusion. Conversely, in our study, CD16+ lymphocyte levels did not differ significantly between the two groups at any time during the observation period. Administration of fentanyl did not suppress, but instead increased NKCC in an in vivo study [24]. The controversial results may be partly attributable to the differences in study design (in vivo versus in vitro study) and study subjects (human versus animal study). Additionally, in clinical studies, the type of anesthesia (volatile or intravenous anesthetic agents) [25] and degree of catecholamine release induced by surgical stress [26] may have some effects on fentanyl-induced immune modulation.

Several studies have assessed the immunomodulatory effects of NSAIDs. Kundu et al. [27] reported that indomethacin and celecoxib increased NKCC by down-regulating the major histocompatibility complex-1 expression in a syngeneic murine model of metastatic breast cancer. In another study, Benish et al. [28] inoculated rats with syngeneic tumor cells and treated them with cyclooxygenase (COX)-1 inhibitors, COX-2 inhibitors, a beta-blocker, or a combination of a COX-2 inhibitor and a beta-blocker, 1 h after which they underwent laparotomy. Combined treatment with the COX-2 inhibitor and a beta-blocker abolished post-operative persistence of the lung tumor and caused significant attenuation of the surgery-induced suppression of NKCC.

In the present study, transient suppressive effects on NKCC were observed in group F compared to that in group N. This indicates that flurbiprofen may be preferable for use as a postoperative analgesic as compared to fentanyl in patients requiring prolonged sedation in the ICU, due to the transient adverse effects of fentanyl on NKCC.

We found no significant differences in cytokine levels between the two groups throughout the study period. Bastami et al. [29] showed that fentanyl did not inhibit cytokine release in an in vitro study. In contrast, Yardeni et al. [22] reported a diminished proinflammatory cytokine response during the perioperative period. In terms of the effects of NSAIDs on cytokine production, Inaoka et al. [30] reported that NSAIDs directly inhibited cytokine production. Many factors, such as surgical stress, anesthetic agents, and method of pain relief after surgery, could modulate cytokine production or release during the perioperative period; hence, further study is necessary to clarify the effects of analgesic agents on cytokine release during the perioperative period.

There are several limitations to the current study. First, we did not conduct an experiment to demonstrate whether fentanyl directly suppresses immunocompetent cells. Second, NKCC on POD1 in group F was significantly depressed compared to the level in group N, but there were no differences between the two groups on POD2. The precise mechanism of NKCC recovery on POD2 in group F was not clear. One possible mechanism is, as reported by Nelson et al. [31], that morphine suppresses splenic NKCC at 1, 6 and 12 h after morphine (15 mg/kg) injection, with recovery at 24 h after the injection. This observation suggests that our results could reflect the time course of changes in immunomodulatory effects of analgesic agents. This speculation was confirmed by the previous study [31]. Third, the total dose of propofol administered in the ICU, although not significantly different between the two groups, tended to be higher in group N. However, based on the results of an in vivo animal study [32], which suggested that propofol has no effects on NKCC in whole blood, we believe that the difference did not affect the present results. In both groups, fentanyl that was administered during the operation might have had some effects on the results of our study. However, the study from Yardeni et al. [22] showed that the total intraoperative dose of fentanyl according to weight (0.012 ± 0.005 mg/kg in group N; 0.012 ± 0.007 mg/kg in group F) would have little effect on NKCC. Although renal failure (an increase of serum creatinine levels or blood urea nitrogen, and decrease of estimated glomerular filtration rates) was not observed in any of the patients in group N in this study, it is not recommended to use flurbiprofen as an analgesic agent in patients with renal failure. In addition, it is uncertain whether prolonged low-dose infusion of flurbiprofen is safe in critically ill patients. Thus, further study is necessary to identify the safety of prolonged administration of flurbiprofen in critically ill patients.

There have been some reports showing that mu opioid agonists suppress NK cell activity [8]. Hence, it is possible that remifentanil used during the operation might have had some effects on our results. Cronin et al. [33] examined the effect of low doses of remifentanil (0.02–0.04 μg/kg/min) on NK cell number and function in human volunteers, and found that an 8-h infusion of remifentanil did not affect NKCC in normal volunteers. Thus, we believe that the remifentanil used in this study did not affect our results.

In conclusion, we investigated the comparative effects of the long-term administration of fentanyl and flurbiprofen on the immune system. Fentanyl transiently decreased NKCC 24 h after the start of its administration, in comparison to flurbiprofen, the difference disappearing 24 h later. Our results suggest that some analgesic agents may have transient suppressive effects on NKCC in patients requiring prolonged postoperative sedation (over 24 h) in the ICU.

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