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Report

Effect of Remifentanil-Based Anesthesia on Perioperative Phagocytic Function of Human Monocytes

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Although morphine-induced modulation of immune cells has been well studied, modulation of immune cells by fentanyl and remifentanil, the latter of which has been used in recent anesthesia procedures, has not been well-studied. Our aim was to identify the effects of fentanyl and remifentanil on phagocytosis and respiratory burst in leucocytes in *in vivo* and *in vitro* studies. In the *in vivo* study, twelve patients were assigned to receive fentanyl-based anesthesia (fentanyl group, n=6) or remifentanil-based anesthesia (remifentanil group, n=6). Blood samples were obtained from before anesthesia to 30 min after anesthesia in each group. In the *in vitro* study, blood samples were obtained from three healthy volunteers and incubated with various blood concentrations of fentanyl or remifentanil (from 0.3 ng/mL to 9 ng/mL). Phagocytic activity (percentage of phagocyting cells) and respiratory burst activity (percentage of cells producing oxygen radicals) were analyzed. In the *in vivo* study, phagocytosis was suppressed only before incision in the fentanyl group whereas suppression of phagocytosis was continued until emergence from anesthesia in the remifentanil group. In the *in vitro* study, incubation with fentanyl or remifentanil tended to enhance phagocytic function of monocytes and had no dose-dependent effect over various concentrations of fentanyl or remifentanil, respectively. Remifentanil-based anesthesia suppressed the phagocytic function of monocytes during its administration.

Key words immunosuppression, monocyte, phagocytosis, fentanyl, remifentanil

INTRODUCTION

Opioids are the gold standard to treat acute and chronic pain, despite having several adverse effects. In recent years, opioid receptors on immune cells, and the interaction between the immune system and opioids have received considerable attention. Roy and colleagues demonstrated that morphine suppressed innate and adaptive immune function *in vitro* and *in vivo*.[1,2] Phagocytosis and respiratory burst are the first line of defense against microorganisms. Morphine and other selective opioid receptor agonists suppressed murine macrophage phagocytosis of Candida albicans.[2,3] Morphine suppressed the phagocytic function of rat peritoneal macrophages like other opioid receptor agonists (DAMGO), and like delta-opioid receptor agonists (meto-enkepharine).[4,5] Human monocytes incubated with morphine presented depression of chemotaxis.[6] Also, morphine modulated phagocytosis by peritoneal macrophages obtained from mu-opioid receptor knockout mice.[7] To date, studies on the interaction between the immune system and opioids have focused on morphine.[1,2] However, in the perioperative period, synthesized opioids such as fentanyl and remifentanil are frequently administered. Welters *et al*., demonstrated that morphine suppressed complement and Fcgamma receptor expression on neutrophils, while fentanyl did not have these suppressive effects.[8] A study on volunteers who received administration of fentanyl demonstrated that respiratory burst of polymorphonuclear cells (PMNC) was not suppressed.[9] An *in vitro* study demonstrated that when human neutrophils were incubated with fentanyl, remifentanil, or alfentanil, neutrophil respiratory burst was not inhibited.[10] The effect of remifentanil on innate immune function has not been fully studied.

In a retrospective study, we found that the incidence of surgical site infection (SSI) in patients who received remifentanil-based anesthesia was significantly higher than that in patients who received fentanyl-based anesthesia in colorectal surgery.[11] There may be a difference in immune modulation induced by fentanyl and that by remifentanil. The reason for the difference in immune function between fentanyl-based anesthesia and remifentanil-based anesthesia has not been elucidated. We conducted *in vivo* and *in vitro* studies on the influence of remifentanil or fentanyl on human leucocytes phagocytosis and respiratory burst.

MATERIALS AND METHODS

***In Vivo* Study** Twelve female patients who were scheduled to undergo laparoscopic ovarian cystectomy were enrolled in this single blind study. This study was approved by the ethics committee of Musashikosugi Hospital Nippon Medical School. Written informed consent was obtained from the patients. Patients were assigned to one of two groups: patients in the fentanyl group received fentanyl-based anesthesia (n=6),

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and patients in the remifentanil group received remifentanil-based anesthesia (n=6) during surgery. Patients with febrile symptom and who taking medication for pain such as steroids or nonsteroidal anti-inflammatory drugs were excluded from this study.

Anesthesia Procedure and Blood Sampling In the operating room, the left antecubital vein was secured for perioperative infusion and the right antecubital vein was secured for blood sampling. An epidural catheter for postoperative pain management was placed at the Th12/L1 interspace. Patients in the remifentanil group received a bolus of 1 μg/kg remifentanil followed by continuous infusion of remifentanil 0.5 μg/kg/min, and patients in the fentanyl group received a bolus of 2 μg/kg fentanyl at the induction of anesthesia. Three minutes after the start of remifentanil administration or bolus administration of fentanyl, propofol 2 mg/kg was administered. Administration of sevoflurane was started via a tight-fitting face mask. Rocuronium 0.7 mg/kg was administered to facilitate intubation. In the remifentanil group, after intubation, the rate of remifentanil was decreased to 0.25 μg/kg/min. The concentration of sevoflurane was set at 1.0%. Just before skin incision, the rate of remifentanil was increased to 0.5 μg/kg/min in the remifentanil group, and a bolus of fentanyl 1 μg/kg was administered to the patients in the fentanyl group. The concentration of sevoflurane was increased to 1.5%.

Anesthesia was maintained using sevoflurane 1.2-2.0% and continuous infusion of remifentanil 0.25-1.0 μg/kg/min in the remifentanil group or administration of intermittent bolus, 1 μg/kg of fentanyl, in the fentanyl group. Administration of remifentanil was terminated at the end of surgery. The concentration of sevoflurane was set to zero. After the patient awoke, the trachea was extubated. After a blood sample was obtained following emergence from anesthesia, continuous infusion of 0.25% levobupivacaine 5 mL/h was started.

Obtaining Blood Samples Five mL of venous blood was sampled before anesthesia induction (Baseline), just before skin incision (Before incision), 60 min after skin incision (During surgery), just after the extubation (Emergence), and 30 min after extubation (After anesthesia). Blood samples were kept in heparinized tubes at 4°C until measurement.

***In Vitro* Study**

Blood Sampling To investigate the dose-dependent effect of remifentanil and fentanyl, we conducted an *in vitro* study using blood samples from volunteers. After informed consent, 10 mL of venous blood was obtained in a heparinized tube from 3 healthy male volunteers. None of the donors had a history of infection or allergy; none were smokers or receiving immunosuppressive drugs.

Fentanyl and remifentanil were obtained from Daiichi Sankyo (Tokyo, Japan) for this investigation. Whole blood 500 μL was incubated with either fentanyl or remifentanil at a concentration of 0 (control), 0.3 ng/mL, 1 ng/mL, 3 ng/mL, or 9 ng/mL for 10 min.

Blood samples were prepared for measurement of phagocytosis and respiratory burst.

Measurement of Phagocytosis and Respiratory Burst

Phagocyte Index Phagocytic activity was measured by the PHAGOTEST kit (Glycotope Biotechnology GmbH, Hedelberg, Germany). Heparinized whole blood was incubated with FITC-labelled *Escherichia coli* (*E. coli*) bacteria at 37°C. Heparinized whole blood was incubated with FITC-labelled (*E. coli*) bacteria and kept on ice as a negative control. Phagocytosis was stopped by placing the sample on ice. Erythrocytes were removed. The DNA was stained just prior to flow cytometric analysis, and aggregates of bacteria or cells were excluded. Cells were analyzed by flow cytometry using the blue-green excitation light (488 nm argon-ion laser). The percentage of cells having performed phagocytosis (granulocytes and monocytes) was analyzed and expressed as the phagocyte index.

Respiratory Burst The phagoburst index was measured by the PHAGOBURST Kit (Glycotope). The kit contains *E.coli* bacteria as particulate stimulus, the protein kinase C ligand phorbol 12-myrisate 13-acetate (PMA) as high stimulus and chemotactic peptide N-formyl-MetLeuPhe (fMLP) as low physiological stimulus, dihydrofhodaminer (DHR) 123 as a fluorogenic substrate and necessary reagents. Heparinized whole blood was incubated with various stimuli at 37°C, and a sample without stimuli served as a negative background control. Upon stimulation, granulocytes and monocytes produce oxygen metabolites (super oxide anion, hydrogen peroxide, hypochlorous acid) that destroy bacteria inside phagosomes. Formation of reactive oxidants during the oxidative burst can be monitored by the addition and oxidation of DHR123. The reaction was stopped by lysing the solution. DNA was stained to exclude aggregation artifacts. The percentage of cells having produced reactive oxygen radicals was then analyzed and expressed as the phagocyte index.

Flow Cytometry Cells were analyzed by a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) flow cytometer using blue-green excitation light (488 nm argon-ion laser). During data acquisition, a "live" gate was set in the red fluorescence histogram on those events that have at least the same DNA content as a human diploid cell (i.e., exclusion of bacteria aggregates having the same scatter light properties as leucocytes). Alternatively, bacteria can be excluded by using fluorescence triggering in the FL2 or FL3 channel. 10,000-15,000 leucocytes per sample were collected. Neutrophils and monocytes were identified according to their physical characteristics on the SSC/FSC dot plot. Phagocytosis and respiratory burst were analyzed in each population (neutrophils and monocytes) and expressed as the percentage of positive events with green fluorescence.

Statistics

***In Vivo* Study** The phagocyte index and phagoburst index at each time point were compared in each group and change from the baseline value was compared between groups.

The absolute change in phagocyte index as well as phagoburst index was calculated as follows:

Change in phagocyte index (before incision)
= Phagocyte index (before incision) - Phagocyte index (baseline) (1)

Change in Phagoburst index (before incision)
= Phagoburst index (before incision) - Phagoburst index (baseline) (2)

The significance of the difference in changes from baseline value between groups was analysed using the Brunner-Munzel permutation test.

The phagocyte index and Phagoburst index at each time point were compared with the baseline value within the respective group using the studentized nonparametric paired t-test which performs a two-sample studentized permutation test for paired data. The number of permutations for the stu-

dentized permutation test was 1000.

***In Vitro* Study** The values of the Phagocyte index and Phagoburst index at the respective blood concentrations were compared between groups, and the significance of the difference among the respective blood concentrations in each group were compared using the studentized nonparametric paired t-test.

All statistical analyses were performed using lawstat package in R (R project). Significance was set at $p<0.05$. The precise program is described in the following:

https://rdrr.io/cran/nparcomp/man/npar.t.test.paired.html

RESULTS

***In Vivo* Study** All patients underwent surgery with no remarkable problem. Patient characteristics are summarized in Table 1. There were no changes in phagocytosis and respiratory burst in neutrophils at any time point (data not shown).

Phagocytic Activity Figure 1 shows changes in the phagocyte index over time. In the fentanyl group, the phagocyte index before incision was significantly lower than that at baseline ($p<0.05$), whereas in the remifentanil group, the phagocyte indices before incision, during surgery and at emergence were significantly lower than the respective baseline value ($p<0.05$ each). The phagocyte index after anesthesia was significantly higher than the baseline value in the remifentanil group. There was a significant difference in changes from baseline in the phagocyte index during surgery between the remifentanil group and fentanyl group ($p=0.039$, Fig. 2).

Respiratory Burst Activity There was no significant difference in respiratory burst activity over time neither in the remifentanil group nor in the fentanyl group (data not shown). In the fentanyl group, the phagoburst index during surgery tended to be lower than that at baseline although the difference was not significant ($p=0.06$). There were no significant differences in changes from baseline in phagoburst index between the fentanyl group and remifentanil group (data not shown).

***In Vitro* Study**

Phagpcytic Activity Measurements were successfully performed in all samples. Table 2 presents the values of phagocyte index and phagoburst index when whole blood from volunteers was incubated with various concentrations. In the fentanyl group, the phagocyte indices upon incubation with 0.3, 1, or 9 ng/mL were significantly higher than that of the control. Although the phagocyte index upon incubation with 1 ng/mL was significantly higher than that upon incubation with 0.3 ng/mL in the fentanyl group, there was no dose-dependent effect of fentanyl. In the remifentanil, the phagocyte indices upon incubation with 3 or 9 ng/mL were significantly higher than control value, and were significantly higher than that upon incubation with 1 ng/mL; however, there was no significant difference in phagocyte index upon incubation with 3 ng/mL or 9 ng/mL. The phagocyte index upon incubation with 3 ng/mL of remifentanil was higher than that of fentanyl.

Respiratory Burst Activity In the fentanyl group, the phagoburst index upon incubation with 0.3 ng/mL tended to be lower than the control value in the fentanyl group. In the fentanyl group, the phagoburst indices upon incubation with 1, 3, and 9 ng/mL were significantly higher than that upon incubation with 0.3 ng/mL; however, those values were not significantly different from the control (Table2). In the remifentanil group, the phagoburst indices upon incubation with 1 ng/mL and 9 ng/mL were significantly higher than the control value; however, there was no dose-dependent effect.

Table 1. Characteristics of the Patients in the Remifentanil and Fentanyl Groups

	Remifentanil (n=6)	Fentanyl (n=6)	P value
Age (y)	35 ± 4.7	34 ± 4.7	0.62
Height (cm)	155 ± 3.0	154 ± 3.0	0.84
Weight (Kg)	49 ± 3.1	56 ± 8.9	0.27
Opioid until incision (μg/kg)	14.4 ± 0.25	3.6 ± 0.75	<0.0001
Opioid until 60 min after incision (μg/kg)	37.7 ± 2.2	6.3 ± 2.2	<0.0001
Total amount of opioid (μg/kg)	54.9 ± 4.6	7.7 ± 4.6	<0.0001
Sevoflurane at 60 min after incision (%)	1.2 + 0.1	1.75 + 0.1	0.002

Data are expressed as mean ± SD

DISCUSSION

In the present *in vivo* experimental study, we demonstrated that administration of fentanyl or remifentanil decreased phagocytic function. In the fentanyl group, suppression of phagocytic function was observed only before incision, whereas suppression was continued until emergence from anes-

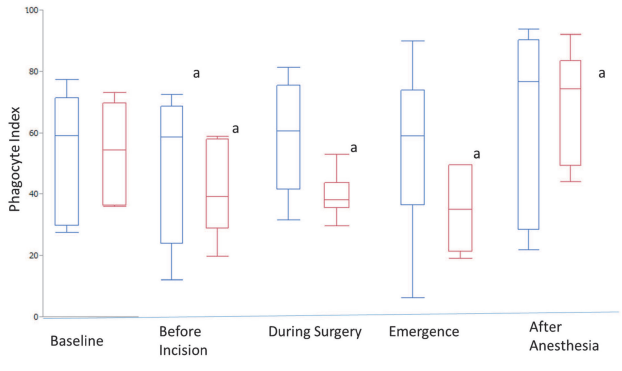

Fig. 1. Absolute Value of the Phagocyte Index Over Time in the Fentanyl (Blue Box) and Remifentanil (Red Box) Groups

The 5 horizontal lines in each box represent the minimum value, 25th percentile, median, 75th percentile and maximum values. a: $p<0.05$ versus baseline in the respective group.

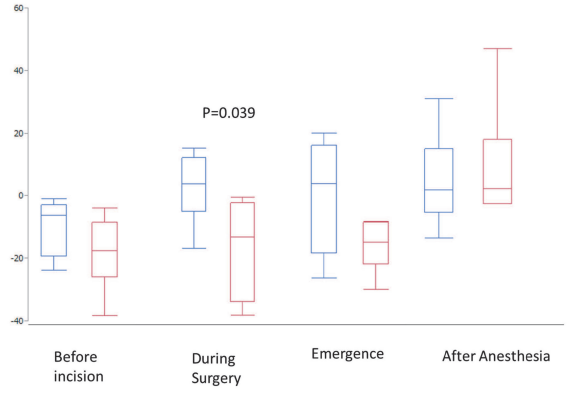

Fig. 2. Absolute Change in the Phagocyte Index from Baseline Over Time in the Fentanyl (Blue Box) and Remifentanil (Red Box) Groups

There was a significant difference between the fentanyl and remifentanil groups ($p=0.039$).

Table 2. Phagocyte Index and Phagoburst Index

	Phagocyte index		Phagoburst index	
	Fentanyl	Remifentanil	Fentanyl	Remifentanil
Control	59.0 (63.0-84.5)	59.0 (63.0-84.5)	52.5 (29.5-57)	52.5 (29.5-57)
0.3 ng/mL	66 (59.0-94.0) a	80.0 (53.0-96.0)	44.0 (17.0-56.0)	48.0 (31.0-59.0)
1 ng/mL	70.0 (66.0-98.0) a, b	65.0 (55.0-90.0)	53.0 (19.0-62.0) b	57.0 (34.0-57.0) a
3 ng/mL	65.0 (58.0-87.0)	82.0 (80.0-90.0) a,d,c	57.0 (21.0-58.0) b	50.0 (23.0-61.0)
9 ng/mL	81.0 (63.0-98.0) a	77.0 (67.0-90.0) a,c	56.0 (21.0-63.0) b	57.0 (31.0-60.0) a

This *in vitro* study was performed using blood samples from three adult volunteers.
Data are expressed as median(range) (n=3).
a: $p<0.05$ versus control, b: $p<0.05$ versus 0.3 ng/mL, c: $p<0.05$ versus 1 ng/mL, d: $p<0.05$ versus Fentanyl

thesia in the remifentanil group. As shown in previous studies, we found no changes in phagocytosis and respiratory burst in granulocytes.[8,9] Although several studies demonstrated that administration of morphine suppresses phagocytosis and chemotaxis of neutrophils and monocytes,[3,4,5] there are few studies demonstrating the effects of fentanyl as well as remifentanil on phagocytic function and respiratory burst. The main finding in the present study is that suppression of phagocytosis recovered during surgery in the fentanyl group, whereas no recovery was found in the remifentanil group until after anesthesia. Recovery of phagocytosis was found just after emergence from anesthesia in the remifentanil group. In the present study, remifentanil was continuously administered whereas fentanyl was intermittently administered. At the end of surgery, infusion of remifentanil was terminated. In general, the blood concentration of remifentanil is around 1-20 ng/mL in remifentanil-based anesthesia, whereas in fentanyl-based anaesthesia, it is kept at around 1-2 ng/mL.[12] Remifentanil undergoes rapid hydrolysis by blood esterase and becomes undetectable within 1 hr after termination.[12] At the time of sampling after anaesthesia, the blood concentration of remifentanil may have been nearly zero. Fentanyl is metabolized in the liver and elimination of fentanyl is stopped in the sampling tube, whereas the level of remifentanil in the sampling tube may have already declined even though the sample was kept at 4°C. The blood samples were subjected to the phagotest and phagoburst approximately 4 h after the end of surgery. Although the blood concentration of fentanyl in the sampling tube reflected that in the patients during the study, that of remifentanil in the sampling tube may have been lower than the blood concentration at the time of blood collection. Again, phagocytic activity in the remifentanil group did not recover until after the anesthesia. The effect of opioid on the immune system is mediated through a direct effect on mu-opioid receptors on immune cells,[13,14] and an indirect effect through mu-opioid receptors in the central nervous system and through the hypothalamic-pituitary-adrenal (HPA) axis.[15,16] In the present study, the blood concentration of remifentanil in the sample obtained after anesthesia may have been zero, whereas remifentanil in the samples obtained before incision, during surgery and emergence may have been detectable even though its concentration was lower than at the time of sampling. The precise mechanism of continuing suppression of phagocytosis in the remifentanil group could not be determined from the results of the *in vivo* study.

The direct effect of each drug on opioid receptors on monocytes may be influenced by its blood concentration, we conducted an *in vitro* study using blood samples from volunteers. Blood was incubated with various concentrations of each drug from a relatively low (0.3 ng/mL) to high concentration (9 ng/mL). The drug concentrations were approximately the same as in the clinical setting. In the fentanyl group, although the level of phagocytic function upon incubation with 1 ng/mL of fentanyl was greater than that upon incubation with 0.3 ng/mL, the levels of phagocytic function upon incubation with 3 ng/mL and 9 ng/mL were equivalent to that upon incubation with 0.3 ng/mL. Suppression of phagocytic function was not observed at any blood concentration of fentanyl and phagocytic function tended to be promoted. There was no dose-dependent effect in modulation of phagocytic function in fentanyl. In the remifentanil group, the levels of phagocytic function upon incubation with 3 and 9 ng/mL were higher compared with the control level and compared with that upon incubation with 1 ng/mL. However, the equivalent levels of phagocytic function between 3 ng/mL and 9 ng/mL indicated that there is no dose-dependent modulation. The *in vitro* study by Bolard, *et al*., indicated that although there was a tendency of fentanyl-induced inhibition of phagocytosis in monocytes, the suppressive effect was small and not dose-dependent.[17] The results of the present *in vitro* study suggest that the direct effect of fentanyl or remifentanil on immune cells may be small and not dose-dependent. The direct effects of fentanyl and remifentanil on the phagocytic function of monocytes may be small.

Filipczak-Bryniarska, *et al*., demonstrated that treatment of mice with an opioid (fentanyl, morphine and methadone) led to significant reduction of hemolytic plaque formation by splenocytes and decreased sheep red blood cell (SRBC)-antibody presenting response by monocytes/macrophages.[18] This reduction may enhance reduced phagocytosis under the presence of opioid or reduction in antigen-presenting effect. This indicates that the residual direct effect of opioid on mu-opioid receptors of donor macrophages, in which the opioid does not have an indirect effect in the recipient, is immunosuppressive.[18,19] The results of the present study coincide with this model. Filipczak-Bryniarska, *et al*. found that opioid treatment stimulated the formation of oxygen radicals by macrophages. In the present study, there was no immune enhancement neither by fentanyl nor by remifentanil.

There is another mechanism that could explain the findings in the present study and that in the studies by Filipczak-Bryniarska *et al*. Mature blood cells are produced at a rate of more than one million cells per second in an adult human.[20,21] Under homeostatic conditions, hematopoietic stem cells from which mature blood cells are derived stay in the G0 phase of the cell cycle. In the present study and the study by Filipczak-Bryniarska, *et al*., there remains a possibility of an indirect effect of fentanyl or remifentanil through differentiation of monocytes from hematopoietic stem cells. An *in vitro* study using a macrophage cell line that resembles a macrophage pro-

genitor cell indicated that administration of morphine inhibited proliferation of the macrophage cell line.[22] Roy *et al*., indicated the possibility that morphine treatment decreases the proliferative capacity of macrophage progenitor cells.[23] Another *in vitro* study using human macrophage cells demonstrated that incubation with morphine inhibited differentiation of monocytes to macrophages.[24] Opioid receptor-deficient mice had increased numbers of progenitor cells that differentiated to granulocytes and monocytes/macrophages.[25] Also, opioid-addicted patients have a reduced number of circulating progenitor cells.[26] These phenomena indicate that the continuous use of remifentanil in the present *in vivo* study may have affected the differentiation of progenitor as well as stem cells during surgery and resulted in an increased number of immature cells that do not possess sufficient phagocytic ability. In our previous study, we found that the use of remifentanil during surgery affected the number of neutrophils.[27] In the present study, continuous infusion of remifentanil may have reduced the number of monocytes that are able to respond to infection.

In conclusion, remifentanil administration suppressed the phagocytic function of monocytes. Further studies are required to identify the reason for this mechanism.

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Conflict of interest The authors declare no conflict of interest.

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