Low-dose remifentanil infusion does not impair natural killer cell function in healthy volunteers


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Background. Mu opioid agonists suppress natural killer (NK) cell activity in animal models. Studies in human volunteers, however, have yielded conflicting results, with morphine suppressing and fentanyl increasing NK cell activity. This study evaluated the effect of a constant 8-h infusion of remifentanil on NK cell number and function in human volunteers.

Methods. After IRB approval and informed consent was obtained, 10 healthy volunteers underwent an 11 pm to 7 am infusion of saline, and at least 1 week later an infusion of 0.02–0.04 μg kg⁻¹ min⁻¹ remifentanil. Blood was collected at 7 am for measurement of NK cell cytotoxicity using a ⁵¹Cr release assay and measurement of NK cell number using fluorescent flow cytometry.

Results. Median and range of the total NK cell cytotoxicity (KU ml⁻¹) was 745.0 (498.3–1483.6) on the control morning and 818.6 (238.5–1454.5) on the morning following the remifentanil infusion. Neither the number of NK cells ml⁻¹ (2.5×10⁵ (1.4×10⁵–4.2×10⁵) vs 2.7×10⁵ (1.1×10⁵–4.4×10⁵)) nor the cytotoxicity per 1000 NK cells (KU 1000 NK cells⁻¹) (3.0 (1.8–5.2) vs 2.9 (0.9–6.7)) changed between the control and remifentanil conditions.

Conclusions. An 8-h infusion of remifentanil did not affect NK cell activity in normal volunteers. This result differs from previous findings of morphine-induced NK cell activity suppression and fentanyl-induced NK cell activity enhancement in normal volunteers.

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Opioids suppress the immune system,¹ including natural killer (NK) cells,² a subset of lymphocytes that defend against intracellular viral and bacterial infections and perform immunosurveillance for cancer cells. Opioid-induced NK cell suppression is a potentially important, but poorly understood, opioid side-effect in surgical patients. The clinical relevance of opioid-induced immune suppression is unclear because of inconsistencies between the results from studies performed in animal models and those performed in human subjects.

In rats, equipotent systemically administered doses of morphine, fentanyl, and sufentanil cause similar reductions of NK cell function.² Recently, remifentanil has also been shown to suppress NK cell function in the rat.⁵ In studies performed in healthy human volunteers, however, the effects of opioids on NK cell activity are inconsistent. Morphine suppresses NK cell activity in a dose-dependant manner.⁴ In contrast, fentanyl increases NK cell number and cytolytic function.⁶ The effect of remifentanil on NK cell activity has not been investigated in humans and is the subject of this study.

Methods

The Institutional Review Board of the Hershey Medical Center approved this study, and informed consent was obtained from all study participants. Healthy volunteers with no history of alcohol or drug abuse, and taking no medications other than oral contraceptives were enrolled. Specific exclusion criteria were a history of chronic opioid use or opioid intolerance, respiratory disease, sleep disorders, or any signs of current or frequent infections.

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Remifentanil titration
At least 2 days before the collection of data on NK cell number and function, subjects underwent titration of remifentanil to determine the dose that evoked a respiratory depressant effect (defined below) and to exclude subjects who experienced nausea or dysphoria. While subjects rested supine in a well-lighted room, an i.v. infusion of saline was initiated. Ventilatory frequency and arterial haemoglobin oxygen saturation (measured using pulse oximetry) were observed for 30 min. Mean of the values obtained at 20, 25, and 30 min were determined and these served as the baseline. A remifentanil infusion was then initiated at 0.02 μg kg⁻¹ min⁻¹ and increased to 0.04 μg kg⁻¹ min⁻¹ after 30 min. Ventilatory frequency and arterial haemoglobin oxygen saturation were monitored every 5 min for 30 min, and the means of the 20-, 25-, and 30-min values were determined. If the ventilatory frequency fell below 75% of baseline or the oxygen saturation fell below 97% of baseline, the infusion rate was reduced in 0.01 μg kg⁻¹ min⁻¹ increments until the ventilatory frequency and oxygen saturation were greater than or equal to these thresholds.

Experimental design
All subjects first underwent a control (saline) infusion and 1–3 weeks later a remifentanil infusion, with the subject blinded to the treatment order. For each infusion, the subject spent two consecutive nights in the General Clinical Research Center (GCRC). The first night was an acclimatization night. The purpose of this night was to ensure that subjects received normal sleep time on the night before data collection and to reduce the likelihood of psychological stress as a result of the unfamiliarity with the study environment. Subjects were admitted to the GCRC at 10.30 pm. The lights, radio, and television were turned off at 11 pm. By 8.15 am the following morning, subjects were discharged and were asked to maintain their normal daytime activities and avoid alcohol.

Subjects returned to the GCRC at 9 pm for the subsequent saline or remifentanil treatment night. On this night, an i.v. catheter was placed in the proximal forearm and an infusion of saline was initiated at 80 ml h⁻¹. Exactly the same protocol described for the acclimatization night was followed on the treatment night, except that an i.v. catheter was placed and a saline or remifentanil infusion was initiated at the predetermined rate at 11 pm and continued until 7 am. Oxygen saturation was monitored throughout the infusions. At 7 am the infusion was discontinued and blood was drawn from the i.v. catheter for determination of NK cell function and number. All subjects were judged ready for discharge by 9 am.

Assay of NK cell cytolytic function
Six millilitres of blood was collected into a Vacutainer™ CPT® tube (Becton-Dickinson, Franklin Lakes, NJ). Lymphocytes and monocytes were separated from the other cellular elements by centrifugation at 1500–1600 g for 20 min at 22°C, washed twice in RPMI 1640 media supplemented with 10% heat-inactivated foetal bovine serum, 100 μg ml⁻¹ streptomycin sulphate, 100 units ml⁻¹ penicillin, 50 μM 2-mercaptoethanol, 2 mM glutamine and resuspended to a volume of 6 ml in supplemented RPMI 1640 media. To determine NK cell lytic activity, 100 μl aliquots of these separated cells were placed in a 96-well V-bottom plate in serial 2-fold dilutions (1:1 to 1:128). Into each well, 2000 ⁵¹Cr-labelled K562 cells were added. Into separate wells, an equal volume of 5% sodium dodecyl sulphate (SDS) detergent was added to produce the maximum possible K562 cell lysis (and release of ⁵¹Cr into the supernatant). Spontaneous (minimum) ⁵¹Cr release from ⁵¹Cr-labelled K562 cells in the absence of NK cell exposure was also determined by the addition of an equal volume of supplemented RPMI 1640 media alone. All wells were incubated for 21 h at 37°C in carbon dioxide 5%. After this incubation period, 50 μl of the supernatant from each well was added to 5 ml of ScintiVerse BD Cocktail (Fisher Scientific) and was counted for 1 min by liquid scintillation analysis to determine the counts per minute (cpm) in each sample. Each dilution of separated cells was assayed in triplicate, and the mean of the three values obtained were used in all further analyses. Cytotoxicity for each dilution of NK cells was expressed as the percentage of the specific lysis (achieved by SDS following removing of spontaneous ⁵¹Cr release):

\[
\text{% specific lysis} = \left( \frac{\text{experimental CPM} - \text{spontaneous CPM}}{\text{maximum CPM} - \text{spontaneous CPM}} \right) \times 100
\]

With no compelling reason to choose any specific dilution as being more relevant than any other, we have chosen to include the data from all of the dilutions (1:1 to 1:128), and to describe the cytolytic activity of each blood sample as the total area under that curve, expressed as killing units (KU) (Fig. 1).

Determination of NK cell number and function
Four millilitres of blood was collected in a Vacutainer™ EDTA tube (Becton-Dickinson) to measure complete blood count and differential cell count using an automated system (Sysmex System) by the Hershey Medical Center clinical laboratory. Total white blood cell count was multiplied by the percentage of lymphocytes and percentage of monocytes to determine the number of lymphocytes and monocytes ml⁻¹ of blood. To determine the percentage of lymphocyte-
plus-monocyte mixture that was NK cells, flow cytometry, using a Becton Dickinson FACScan™ flow cytometer, was performed. This was done using BD TriTEST™ CD3 fluorescein isothiocyanate (FITC)/CD16+CD56 phycoerythrin (PE)/CD45 peridinin chlorophyll protein (PerCP), a 3-colour direct immunofluorescence reagent for identifying and determining the percentages and absolute counts of mature human T lymphocytes and NK cells. Multiplying the number of lymphocytes plus monocytes ml−1 of blood by the percentage that was identified as NK cells (CD16+/CD56+) yielded the number of NK cells ml−1 of blood. The cytotoxicity per 1000 NK cells (KU 1000 NK cells ±1) was determined by dividing the total cytotoxicity of the plate (KU) by the total number of NK cells in the plate and multiplying by 1000. The total NK cell function (KU ml−1) was determined by multiplying the killing units NK cell ±1 by the number of NK cells ml−1 of blood.

Statistical analysis

As the data were not normally distributed, Wilcoxon signed rank test was used to test the primary hypothesis that total NK cell cytolytic activity was decreased by remifentanil and to make additional comparisons of NK cell number and function between saline and remifentanil.

The sample size of 12 patients was based on previous studies that demonstrated a significant effect of morphine and of fentanyl on NK cell function in normal volunteers using seven to nine subjects per group.467 This sample size allowed for a 25% attrition rate to achieve a final subject number of at least 9.

Results

Twenty-five volunteers were interviewed by telephone, and 12 of these enrolled in the study. One withdrew after the remifentanil titration visit and another withdrew after the baseline night. There were no adverse events. Only data from the remaining 10 subjects are included in the analysis (Table 1).

No subjects experienced nausea, vomiting, hallucinations, or dysphoria. However, when asked how refreshed they felt in the morning, seven of the 10 subjects felt less refreshed after the remifentanil night than after the previous acclimatization night, while only two of the 10 subjects felt less refreshed after the saline infusion night than after the previous acclimatization night.

Respiratory depression (ventilatory frequency <75% of baseline and oxygen saturation <97% of baseline) from remifentanil was present in three subjects at 0.02 µg kg−1 min−1, 2 subjects at 0.03 µg kg−1 min−1, and five subjects at 0.04 µg kg−1 min−1. All subjects demonstrated some degree of respiratory depression, but one subject did not meet the respiratory depression threshold (Table 2). Remifentanil caused a statistically significant decrease in ventilatory frequency (P=0.0001) and oxygen saturation (P=0.0017 two-tailed paired t-test).

NK cell function

The median (range) number of NK cells, cytolytic function per 1000 NK cells, and total NK cell function are presented

Table 1 Characteristics of study subjects

| Number | 12 enrolled, 10 completed |
| Age median (range) | 23.9 yr (20–35) |
| Gender | 6 males, 4 females |
| Height median (range) | 180 cm (163–193) |
| Weight median (range) | 75 kg (57–98) |

Table 2 Respiratory response to remifentanil infusion

<table>
<thead>
<tr>
<th>Breaths per minute</th>
<th>SpO2</th>
<th>Infusion rate (µg kg⁻¹ min⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>Remifentanil</td>
<td>Saline</td>
</tr>
<tr>
<td>Subject 1</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Subject 2</td>
<td>10</td>
<td>8</td>
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<tr>
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<td>Subject 10</td>
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Fig 1: Representative area under the curve of NK cell lytic activity from one study subject following saline infusion. The serial dilutions of isolated lymphocytes and monocytes containing NK cells are depicted on the x-axis, and the per cent specific lysis of K562 cells on the y-axis.
deprivation, phase of circadian rhythm, and amount of none of these differences were statistically significant. vs cell number but a small decrease in cytolytic activity per seven to nine subjects per group, the current study would studies found an opioid effect on NK cell activity using cell function in human volunteers. Although previous physical activity, all of which influence NK cell activity. morphine and fentanyl. However, an alternative explanation for these varying results is differences in study design. A specific objective of the design of the current study was to control for factors such as psychological stress, sleep deprivation, phase of circadian rhythm, and amount of physical activity, all of which influence NK cell activity. The short half-life of remifentanil enables this study to evaluate the effect of several hours of a stable plasma opioid concentration on NK cells and permits titration of the opioid dose to a modest physiologic target effect. These conditions minimize possible confounding effects of increasing and decreasing opioid concentrations and dose-dependent side-effects such as nausea or dysphoria that might be associated with neuroendocrine effects on NK cells.

Unlike human studies, animal-based studies consistently demonstrate suppression of NK cell activity by opioids, including morphine, fentanyl, and remifentanil. NK cell suppression occurs following systemic or direct central nervous system administration of opioids to rats. Furthermore, microinjection studies have localized the site of action to the periaqueductual grey matter, and injection of subtype selective agonists has demonstrated that only the mu receptor mediates this opioid effect on NK cell activity. As morphine, fentanyl, and remifentanil are all mu agonists that cross the blood–brain barrier, the animal studies do not help explain why these opioids have differing effects on NK cell activity in humans.

Morphine, fentanyl, and remifentanil have different plasma half-lives and metabolites that might help explain their differing effects on NK cell activity measured in vivo and ex vivo. This possibility is especially attractive because of the presence of opioid receptors on many lymphoid cell types. In this study, for example, continued metabolism of remifentanil by cholinesterases in the blood sample before NK cell analysis might have diminished the measured effect of remifentanil on NK cell activity (but not NK cell number).

However, consistent evidence from both animal and human studies cast doubt on this theory. In animals, systemic administration of N-methyl-morphine, an active morphine derivative that does not cross the blood–brain barrier, does not suppress NK cell activity. In humans, the measured effect of systemically administered morphine or fentanyl on NK cells does not depend upon whether the assay is performed in opioid-containing or opioid-free conditions. Therefore, differences in plasma opioid metabolites or opioid concentrations in the blood sample during the NK cell assay are at best incomplete explanations for the reported differences in effect of morphine, fentanyl, and remifentanil on NK cell activity in humans. Rather than a direct effect of opioids or their metabolites on NK cells in the plasma, the evidence from the animal and human studies points toward a neural-immune linkage to explain the effect of opioids on NK cells.

Because of the evidence indicating that the central nervous system is critical in connecting opioids and NK cells, this study aimed to rigorously control the level of opioid activity in the brain. We attempted to establish a uniform degree of central nervous system opioid activity by using the respiratory response as a functional assay. The minimum dose of remifentanil that achieved clear evidence of mu opioid receptor activation was chosen, and the pharmacokinetic properties of remifentanil enabled us to reliably maintain this stable plasma concentration for several hours. Therefore, the reported NK cell effects are not attributable to increasing or decreasing plasma opioid concentrations. These differences in study design might

Fig 2 NK cell number and function following saline or remifentanil infusion. Median and range of the number of natural killer cells (NK cells ml⁻¹), cytotoxicity per 1000 NK cells (KU 1000 NK cells⁻¹), and the natural killer cell activity (KU ml⁻¹) are displayed for the saline and remifentanil conditions.

(Fig. 2). Following remifentanil NK cell total activity increased from 745.0 (498.3–1483.6) to 818.6 (238.5–1454.5), which reflected a small increase from 2.5×10⁵ (1.4×10⁵–4.2×10⁵) to 2.7×10⁵ (1.1×10⁵–4.4×10⁵) in NK cell number but a small decrease in cytolytic activity per 1000 cells from 3.0 (1.8–5.2) vs 2.9 (0.9–6.7). However, none of these differences were statistically significant.

Discussion
The principal finding of this study is the absence of an effect of remifentanil on NK cell number or cytolytic function. This finding is surprising in light of the suppressive effect of morphine and the enhancing effect of fentanyl on NK cell function in human volunteers. Although previous studies found an opioid effect on NK cell activity using seven to nine subjects per group, the current study would have required more than 800 subjects to have 80% power to detect a difference in NK cell activity between the remifentanil and saline conditions. Therefore, the data suggest that if there is a remifentanil effect on NK cell function or number, it is smaller than has been reported for morphine and fentanyl.

It is possible that the effect of remifentanil on NK cells is different from that of the other mu opioid agonists, morphine and fentanyl. However, an alternative explanation for these varying results is differences in study design. A specific objective of the design of the current study was to control for factors such as psychological stress, sleep deprivation, phase of circadian rhythm, and amount of physical activity, all of which influence NK cell activity. The short half-life of remifentanil enables this study to evaluate the effect of several hours of a stable plasma opioid concentration on NK cells and permits titration of the opioid dose to a modest physiologic target effect. These conditions minimize possible confounding effects of increasing and decreasing opioid concentrations and dose-dependent side-effects such as nausea or dysphoria that might be associated with neuroendocrine effects on NK cells.
explain the differences in results among the studies of the effects of opioids on NK cell activity in human volunteers.

Another significant difference between this and previous studies of opioid effect on NK cell activity in humans is the choice of the control group. Previous studies17 compared the NK cell activity immediately before opioid administration (control) to that activity at time points during or following opioid administration, or used a separate group of five healthy volunteers as a control group.6 The current study is the first to use a placebo (saline) infusion and an opioid infusion in the same study subjects, thus allowing the use of paired comparisons at the same time of day following 8 h of inactivity in a recumbent posture.

Because the opioid effect on NK cells is most likely transmitted through a neural-immune interaction, physical and psychological factors influencing subjective experiences are potentially significant. For this reason, effort was directed to acclimatize the subjects in our study to the study environment and to ensure a normal night of sleep before the treatment night. The lowest opioid dose that evoked a clear physiologic response was used in order to avoid common opioid side-effects such as nausea, with concomitant neuroendocrine alterations.18

Using rigorously controlled conditions, this study has found no evidence of a clinically significant effect of a low dose of remifentanil on NK cell activity in human volunteers. Whether remifentanil is different from morphine or fentanyl in this regard or whether the effects reported by others result from differences in study design needs further study. The clinical implication of these investigations remains unclear pending this information, and ultimately a study of opioid effects on NK cell activity in patients rather than normal volunteers.

**Conclusion**

Administration of a dose of remifentanil that causes modest respiratory depression does not result in alteration of NK cell activity or number in human volunteers. This finding suggests that activation of mu receptors in the brain is not sufficient to cause NK cell suppression. Additional studies are required to elaborate this neural–immune interaction and ultimately to determine the clinical significance of this possible opioid side-effect.

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**References**