Low-dose ketamine affects immune responses in humans during the early postoperative period

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Background. Anaesthesia and surgery are associated with impairment of the immune system expressed as an excessive proinflammatory immune response and suppression of cell-mediated immunity that may affect the course of the postoperative period. Addition of anaesthetic agents capable of attenuating the alterations in perioperative immune function may exert a favourable effect on patients' healing. We have assessed the effect of preoperative administration of a sub-anaesthetic dose of ketamine on the mitogen response and production of interleukin (IL)-1β, IL-2, IL-6, and tumour necrosis factor (TNF)-α by peripheral blood mononuclear cells (PBMCs), as well as natural killer cell cytotoxicity (NKCC) in patients undergoing abdominal surgery.

Methods. Seventeen patients admitted for elective abdominal surgery were given ketamine 0.15 mg kg⁻¹ i.v. 5 min before induction of general anaesthesia. Nineteen patients received a similar volume of isotonic saline 5 min before induction of anaesthesia. PBMCs were isolated from venous blood before and 4, 24, 48, and 72 h after operation for IL-1β, IL-2, IL-6, and TNF-α secretion, and NKCC assessment.

Results. Four hours after operation, the cells from patients in the ketamine group showed a significantly suppressed production of IL-6 (P<0.01) compared with controls. The production of IL-2 did not change from that of the preoperation samples. TNF-α secretion was significantly elevated in the control group 4 h after operation (P<0.05).

Conclusions. Addition of small doses of ketamine before induction of anaesthesia resulted in attenuation of secretion of the proinflammatory cytokines IL-6 and TNF-α, and in preservation of IL-2 production at its preoperative level. It is suggested that this anaesthetic may be of value in preventing immune function alterations in the early postoperative period.


Keywords: anaesthetics, i.v., ketamine; immune response; natural killer cells

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The immune system of patients undergoing surgery is affected by both anaesthesia and surgical trauma, with possible sequelae on the postoperative outcome. Altered immune reactions including natural killer (NK) cell activity have been observed after the use of volatile anaesthetics and opioids, such as morphine and large doses of fentanyl.¹–⁴ The in vivo and in vitro effect of volatile anaesthetics on various parameters of the immune system has been reviewed by Homburger and Meiler.¹ Elena and colleagues⁵ have reported a decrease in the absolute number of both leucocytes and lymphocytes in the peripheral blood of mice exposed to repeated administration of sevoflurane anaesthesia. Brand and colleagues⁶ have shown a decrease in circulating NK cells associated with an increase in B cells, CD8-T lymphocytes, interferon (IFN)-γ, IFN-α, tumour necrosis factor (TNF)-α, and...
soluble interleukin (IL)-2 receptor. The cytokine cascade activated in response to surgical trauma consists of a complex biochemical network with diverse effects on the injured host, that is, some components of the immune system, such as proinflammatory cytokines, are stimulated to an excessive degree, whereas other functions, for instance, cell-mediated immunity, are dramatically suppressed. Any significant amplification in the proinflammatory response potentially predisposes the host to undesirable consequences, including hypotension, shock, and even multiple organ failure. Thus, excessive production of proinflammatory cytokines due to anaesthesia and surgery may induce severe inflammation and postoperative complications. In addition, the immune system and the nervous system communicate bidirectionally and it has been suggested that nociception and proinflammatory cytokines play a mutual up-regulatory role; thus, increased production of proinflammatory cytokines may exacerbate pain, and vice versa. Therefore, it is conceivable that effective pain management may affect the immune responses during the postoperative period. An attempt to diminish the deleterious side-effects of the opiates on the immune system could be achieved by addition of drugs with marked analgesic activity capable of attenuating pain stimuli and therefore allowing reduction of the dosage of opiates. Ketamine, a neurotransmitter acting as a N-methyl-D-aspartate receptor antagonist, has been shown to enhance the effect of morphine in control of perioperative pains. Cherry and colleagues have achieved a reduction in morphine dosage and pain score by addition of ketamine, with a direct relationship between ketamine dosage and pain score. Similar results have been observed in patients undergoing knee or abdominal surgery. It has been shown that small doses of ketamine exert analgesic action in the early stages of formation of pain stimuli; therefore, it may be useful in inducing pre-emptive anaesthesia. The question of whether ketamine, in addition to its beneficial action as a pain reliever, may attenuate the immunosuppressive effect of opioids in patients exposed to surgery is of interest to the clinician. Roytlat and colleagues have reported a decrease in serum IL-6, an activator of the inflammatory cytokine cascade, in patients undergoing coronary artery bypass surgery and in women submitted to abdominal hysterectomy who received small doses of ketamine. The present study was designed to assess the immunomodulatory effect of small doses of ketamine on the mitogen response and production of IL-1β, IL-2, IL-6, and TNF-α, by peripheral blood mononuclear cells (PBMC) from patients undergoing abdominal surgery. In addition, the influence of the drug on NK cell cytotoxicity (NKCC) was evaluated. The rationale of the study was to contribute to understanding the role of subanaesthetic doses of ketamine in attenuating the undesirable effect of anaesthesia and surgery on the immune system.

### Methods

#### Patients

The Helsinki Committee of the Soroka Medical Center approved the study that comprised 36 patients undergoing abdominal surgery. The patients were premedicated with diazepam 5–10 mg given orally 90 min before operation and with i.v. administration of midazolam 2–3 mg upon arrival at the operating theatre. Thereafter, the patients were assigned to two groups according to randomized sequences without duplicates. Group A comprised 17 patients who received racemic ketamine 0.15 mg kg⁻¹ i.v. 5 min before induction of general anaesthesia. This dose was chosen according to previous experience with the drug. Group B consisted of 19 patients who received a similar volume of isotonic sodium chloride 5 min before induction of anaesthesia. There was no significant difference between patients from the two groups with respect to age, body weight, type, and duration of surgery and there was no requirement for blood transfusions (Table 1). Anaesthesia was induced by i.v. administration of fentanyl 2–3 μg kg⁻¹, thiopentone 4–6 mg kg⁻¹, and vecuronium 0.1 mg kg⁻¹, and was maintained with nitrous oxide, isoflurane, and addition of fentanyl. The total dose of fentanyl and thiopentone, and the average dose of isoflurane administered to the patients, is given in Table 1. Mean arterial pressure was maintained within 20% of baseline values with isoflurane and fentanyl. The patients received upper body forced-air warming, and the i.v. fluids were warmed to 37°C.

#### Immunological assays

Venous blood samples (15 ml) were collected before surgery and at 4, 24, 48, and 72 h after operation. PBMCs were isolated from heparinized venous blood using histopaque (Sigma) gradient centrifugation. The cells were washed twice in RPMI-1640 medium containing penicillin 1%, streptomycin, and nystatin and supplemented with 10% fetal calf serum (FCS), designated as complete medium (CM). Thereafter, they were suspended in FCS containing dimethyl sulphoxide 10% (DMSO, Sigma) and frozen at −70°C until used. On the day of assay, the cells were thawed quickly,

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Control</th>
<th>Ketamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hysterectomy</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Gastroplasty (SRVG)</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43.5 (29–58)</td>
<td>39.0 (28–55)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/14</td>
<td>4/13</td>
</tr>
<tr>
<td>Duration of operation (min)</td>
<td>113 (7.34)</td>
<td>105 (6.51)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>97.5 (26.3)</td>
<td>95.4 (29.9)</td>
</tr>
<tr>
<td>Fentanyl (μg) (total dose)</td>
<td>387 (46.1)</td>
<td>395 (49.1)</td>
</tr>
<tr>
<td>Thiopentone (mg) (total dose)</td>
<td>353 (29.3)</td>
<td>367 (32.1)</td>
</tr>
<tr>
<td>Isoflurane (average dose)</td>
<td>1–1.5%</td>
<td>1–1.5%</td>
</tr>
</tbody>
</table>
washed three times in CM, and their viability tested by trypan blue dye exclusion was more than 95%.

**IL-1β, IL-2, IL-6, and TNF-α production**

PBMCs (2×10^6) suspended in 1 ml of RPMI-1640 medium supplemented with FCS 5% were incubated for 24 h in the presence of 10 mg ml⁻¹ lipopolysaccharide (Escherichia coli, LPS, Sigma) for evaluation of IL-1β, IL-6, and TNF-α production. For IL-2 production, 2×10^6 PBMCs were suspended in 1 ml of CM and incubated for 48 h with phytohaemagglutinin 1% (PHA-M, Difco). After incubation, the culture media were collected, the cells were removed by centrifugation, and the supernatants were kept at −70°C until assayed for cytokine content.

Cytokine concentration in the supernatants was tested using ELISA (enzyme-linked immunosorbent assay) kits specific for human IL-1β (Biosource International, Camarillo, CA, USA), IL-6 and TNF-α (Pharmingen, San Diego, CA, USA), and IL-2 (R&D systems, Minneapolis, MN, USA), as detailed in the guideline provided by the manufacturers. The detection level of these cytokines in the assays was 30 pg ml⁻¹ for IL-1β, IL-2, and TNF-α, and 15 pg ml⁻¹ for IL-6.

**Mitogen response**

0.1 ml of PBMC suspension (2×10^6 cells ml⁻¹) was aliquoted into each of 96-well plates (flat bottom, Nunc) containing 0.1 ml of CM or PHA (Difco, 2%), concanavalin A (Con A, 10 μg ml⁻¹), or pokeweed mitogen (PWM, Sigma, 20 μg ml⁻¹). Cultures set-up in triplicate were incubated for 3 days. About 0.5 μCi per well of [³H]TDR (methyl-[³H]thymidine, 5 μCi mmol⁻¹, Amersham, UK) was added 18 h before harvesting. Radioactivity was measured with an LKB liquid scintillation counter model 3380.

**NKCC assay**

Cytotoxicity was assessed by a standard chromium specific release assay with the ⁵¹Cr-labelled K562 cell line used as target cells, and PBMC serving as effector cells. The final effector to target (E/t) ratio was 100:1. After 4 h of incubation at 37°C, the supernatants were collected, and the radioactivity was measured using a gamma counter (LKB).

All reactions were carried out in triplicate and the specific ⁵¹Cr release was calculated as described earlier.³

**Statistical analysis**

Data were analysed for each measure using analysis of variance with repeated measures (for time period before and after surgery) and Student’s t-test to compare the difference between the two groups at each individual time point. Probability values of P<0.05 were considered as significant. The results are expressed as means (SEM).

**Results**

**Interleukin production**

**Interleukin-1β**

There was no significant difference in IL-1β production before surgery between the two groups (Table 2). However, in patients from both groups, the IL-1β level increased significantly at 24, 48, and 72 h after surgical intervention.

**Interleukin-2**

The secretion of IL-2 by PHA-stimulated PBMCs from individuals in the control and study groups was similar before surgery. At 4, 24, 48, and 72 h after the operation, IL-2 production decreased significantly in patients from the control group, whereas in the study group it did not differ from that before surgery at any of these time points. The difference between the two groups did not reach statistical significance at any experimental point.

**Interleukin-6**

IL-6 production by PBMCs from patients in the control and study groups was similar before surgery. At 4 h after operation, IL-6 secretion was elevated in the control, but not in the study group. The difference between the results from the two groups was statistically significant (P<0.05). Increased production of IL-6 was found at 24, 48, and 72 h after operation in both groups. However, there was no significant difference between the two groups at the later postoperative times.

Table 2  Cytokine production by PBMCs from patients in the two groups. *P<0.01, **P<0.05 (statistically significant different from the values before operation). ³Statistically significant different from the values in the control group at the same amount of time after operation.

<table>
<thead>
<tr>
<th>Cytokine (mg ml⁻¹)</th>
<th>Controls Before operation</th>
<th>Hours after operation</th>
<th>Ketamine Before operation</th>
<th>Hours after operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>IL-1β</td>
<td>6.6 (0.3)</td>
<td>7.9 (0.8)</td>
<td>8.5 (0.7)*</td>
<td>8.3 (0.8)*</td>
</tr>
<tr>
<td>IL-2</td>
<td>5.3 (0.5)</td>
<td>3.7 (0.5)**</td>
<td>3.4 (0.5)*</td>
<td>3.2 (0.5)*</td>
</tr>
<tr>
<td>IL-6</td>
<td>71.4 (5.4)</td>
<td>111.4 (16.7)*</td>
<td>120.7 (14.1)*</td>
<td>129.5 (14.8)*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>12.5 (0.8)</td>
<td>15.4 (1.3)**</td>
<td>15.0 (1.7)**</td>
<td>16.5 (1.7)**</td>
</tr>
</tbody>
</table>
Tumour necrosis factor-α

TNF-α values before surgery did not differ significantly between the control and the study group. Four hours after operation, the production of TNF-α in the study group was similar to that before surgery, whereas in the control group it was significantly elevated. TNF-α level was higher at 24, 48, and 72 h after operation in both groups.

Mitogen-induced lymphocyte proliferation

The lymphocyte proliferative response to PHA was suppressed in the control group for the first 24 h after operation and was lower, but not statistically significant, in the study group (Fig. 1). Forty-eight hours after operation, the proliferative response to PHA in both groups returned to preoperative values. Con A and PWM-induced proliferation did not change significantly 72 h after operation in both groups (data not shown).

Natural killer cell cytotoxicity

Before operation, NKCC was similar in the control and study group and was suppressed in both groups at 24 and 48 h after surgery (Fig. 2). This cell function partially recovered to preoperative values 72 h after operation.

Discussion

The results of the study show that addition of low-dose ketamine during induction of anaesthesia attenuates the ex vivo enhancement of IL-6 and TNF-α production by patients’ PBMCs, at least at 4 h after operation. Furthermore, ketamine supplementation preserved the pre-operative IL-2 level and PHA-induced cell proliferation up to 24 h. In a previous study, we demonstrated that pre-emptive low-dose ketamine significantly decreased post-operative pain and reduced morphine requirement.

Moreover, attenuation of postoperative pain reduced the suppression of the lymphocyte proliferative response to mitogens and attenuated the proinflammatory cytokine reaction to surgery. These findings are in accordance with those of Roytblat and colleagues, who have reported that patients undergoing cardiopulmonary bypass, who were given small doses of ketamine during induction of anaesthesia, showed significantly lower serum levels of IL-6 during the course of seven postoperative days. Taniguchi and colleagues have shown that ketamine administration dose-independently inhibited hypotension, metabolic acidosis, and cytokine responses when injected with endotoxin. In another study, it was found that addition of low-dose ketamine to fentanyl anaesthesia suppresses IL-6 production at 4–72 h after abdominal hysterectomy. Patients with severe burns given small doses of ketamine with or without fentanyl in the course of patient-controlled i.v. analgesia showed a decrease in serum IL-1, IL-6, and TNF-α.

In a study with rats, it has been found that ketamine improved the survival in those with burn injuries complicated by sepsis, an effect explained by decreased IL-6 production observed in septic animals. Kawasaki and colleagues have carried out in vitro studies with human whole blood and reported a suppressive effect of ketamine on LPS-induced TNF-α, IL-6, and IL-8 production. TNF-α is the first cytokine which stimulates IL-6 and IL-8 production by macrophages. In view of the fact that ketamine also suppressed rhTNF-α-induced IL-6 and IL-8 production, the authors suggested that the decrease in IL-6 and IL-8 is due not only to suppression of TNF-α, but also to a direct inhibitory effect of the drug on the production of these cytokines in whole blood. Since the immune parameters in the present study were determined at fixed time points, it is possible that the reduced cytokine secretion observed during the postoperative period in patients treated with low-dose ketamine may reflect either an attenuated or delayed proinflammatory response.

Fig 1 Proliferative response to PHA by PBMCs from patients of the two groups. Data are expressed as mean (SEM). Asterisks represent a statistically significant difference from values before operation (**P<0.01).

Fig 2 NKCC of PBMCs from patients of the two groups. Data are expressed as mean (SEM). Asterisks represent a statistically significant difference from values before operation (*P<0.05, **P<0.01).
The role of IL-6, TNF-α, and IFN-γ as proinflammatory cytokines is well established. It has been reported that these cytokines may be increased in patients with sepsis, asthma, heart failure, trauma, and burns. The way they participate in both humoral and tissue responses during injury is detailed in the excellent review by Feghali and colleagues. TNF-α and IL-6 are involved in both acute and chronic inflammation, and in the case of polymicrobial sepsis in rats and in horses, IL-6 acts as a mediator of cellular responses. It is therefore suggested that ketamine-induced prevention of IL-6 and TNF-α production may exert a favourable effect on a patient’s recovery. Since IL-2 plays a major role in the regulation of both cellular and humoral inflammatory responses, the ability of ketamine to preserve its secretion at the preoperative level observed in the present study is an additional contribution to avoiding postoperative complications. In the present study, ketamine exerted an effect on PHA-induced proliferation of PBMCs, whereas the other two mitogens examined were not affected. Considering the fact that PHA and Con A activate T cells, whereas PWM activates B cells, it is plausible that ketamine may affect the lymphocyte subpopulations differently.

It appears that ketamine may exert a beneficial effect on the post-surgical immune response via several mechanisms. Acting as an analgesic, it causes alleviation of pain, which by itself is a promoter of proinflammatory cytokine production and suppressor of IL-2 secretion. Therefore, it may be useful in administration of pre-emptive analgesia via suppression of inflammation. The direct suppressive effect of ketamine on proinflammatory cytokine production by PBMCs demonstrated in the present ex vivo study and in other works using serum and whole blood is an additional indicator for its valuable effect. Furthermore, ketamine exerts an anti-inflammatory effect by inhibition of leucocyte reactivity and suppression of increased superoxide anion production by neutrophils after coronary artery bypass grafting. Since proinflammatory cytokines suppress cAMP accumulation in heart cells, the inhibitory effect of ketamine on these cytokines will improve cAMP build-up with a subsequent beneficial effect on cardiac output and arterial pressure.

In conclusion, the results of the study indicate that addition of a small dose of ketamine before induction of anaesthesia induces an attenuation of IL-6 and TNF-α production, both acting as proinflammatory cytokines, and a preservation of IL-2 at its preoperative level. These findings favour the value of ketamine in preventing immune function alterations caused by anaesthesia and surgery.

References

Ketamine and cytokine production


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