Addition of noradrenaline to intrathecal morphine augments the postoperative suppression of natural killer cell activity

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Abstract

Purpose. Intrathecal administration of morphine has been shown to suppress natural killer (NK) cell activity. We tested the hypothesis that combined administration of morphine and noradrenaline would further modify NK cell activity in patients undergoing hysterectomy.

Methods. Thirty female patients were randomly divided into three groups of ten patients each. Groups MN and M received intrathecal morphine (0.5 mg) dissolved in 5 ml of physiological saline with and without 5 µg noradrenaline, respectively. Group C received saline alone. After the intrathecal administration, general anesthesia was induced. Blood samples were withdrawn before and 2 h after surgery and on postoperative days 1, 2, and 7 to determine the NK cell activity, the ratio of T-helper/inducer cells (CD4) to T-suppressor/cytotoxic cells (CD8), the levels of interleukin-6 (IL-6) and interleukin-8 (IL-8), and the plasma concentrations of catecholamines and cortisol.

Results. NK cell activity decreased on postoperative day 1 in groups MN (12.0 ± 2.7%) and M (25.4 ± 9.6%) compared with their respective baseline levels. In group MN, NK cell activity remained lower (23.7 ± 8.0%) on postoperative day 2 than the baseline value before surgery.

Conclusion. Intrathecal administration of morphine causes a decrease in NK cell activity, and its combined use with noradrenaline prolongs the suppression of NK cell activity.

Key words Morphine · Noradrenaline · Spinal anesthesia · NK cell activity

Introduction

Opioids are widely used to alleviate various types of pain and to supplement general anesthesia. Especially, intrathecal opiate analgesia, without the loss of motor function or other sensory modalities, has played an important role in the control of acute and chronic pain. Natural killer (NK) cells, because of their unique ability to recognize and kill tumor cells without processing tumor-specific antigen, are thought to form a primary immune defense mechanism. We have previously shown that intrathecal morphine is useful for the treatment of postoperative pain, but that it causes suppression of NK cell activity [1]. Intrathecal coadministration of morphine and an alpha-adrenergic agonist enhances analgesia [2]. Goto et al. [3] reported that the main mechanism of prolonging spinal anesthesia by added noradrenaline may be a direct effect on the spinal nociceptive system. It is valuable for patients to have enhanced postoperative analgesia and to avoid immunosuppression. Suppression of immune defense mechanisms occurs in the postoperative period [4-6]. Such compromised immunity could affect the postoperative infection rate, healing reactions, and the rate and size of tumor metastasis disseminated during surgery [7].

In order to investigate whether the addition of noradrenaline enhanced postoperative analgesia and prolonged the suppression of NK cell activity by intrathecal morphine, we studied 30 patients undergoing hysterectomy for uterine myoma.

Subjects and methods

With local Ethics Committee approval and informed patient consent, we studied 30 adult patients with uterine myoma who were undergoing elective total hysterectomy. The patients had normal cardiac, renal, and hepatic functions. None had endocrine disorders and none was receiving any opioid. The patients were randomly assigned to one of three groups. Patients in group M (n = 10) received intrathecal morphine (0.5 mg) dissolved in 5 ml of physiological saline, through a spinal needle inserted into L5-4 before induction of general
anesthesia. Group MN (n = 10) received 0.5 mg of morphine plus 5 µg of noradrenaline. Group C (n = 10) received saline alone. After intrathecal administration, general anesthesia was induced with 5 mg·kg⁻¹ thiamylal and 0.12 mg·kg⁻¹ vecuronium for tracheal intubation. Anesthesia was maintained with isoflurane 0.6%–1.5% and nitrous oxide 66% in oxygen. Vecuronium was given as needed during surgery. None of the patients received a blood transfusion.

After the patients’ recovery from general anesthesia, pain scores, according to a verbal-description pain scale, were examined. Postoperative pain was assessed by one of three observers, all of whom were unaware of which group the patient belonged to. The observer questioned the patient and asked them to breathe deeply, cough, and move about. Pain was rated on a scale of 0 to 4 (0, pain free-with movement or coughing; 1, minimal discomfort on movement or coughing; 2, comfortable at rest, moderate pain on movement or coughing; 3, discomfort at rest, considerable pain with movement or coughing; 4, severe pain, even at rest).

For postoperative analgesia, nonsteroidal antiinflammatory drugs such as diclofenac sodium and indomethacin were used; no opioids were used.

Blood samples were withdrawn before surgery at, around 9 a.m., for baseline values, and then 2 h after surgery and on postoperative days 1, 2, and 7, to determine blood NK cell activity, the CD4/CD8 ratio, interleukin (IL)-6 and IL-8 levels, and plasma noradrenaline, adrenaline, and cortisol concentrations. No patients received exogenous catecholaminergic medications during or 7 days after surgery.

NK cell activity was measured against K-562 target cells in a chromium-51(⁵¹Cr) release assay, in which ⁵¹Cr-labelled target cells (5 × 10⁶) of the human erythroleukemic cell line K-562 were mixed with different concentrations of mononuclear cells from blood samples of the patients, for use as effector cells, to obtain effector-to-target cell ratios of 100:1, 50:1, 25:1, and 12:1. Cell suspensions were incubated for 4 h at 37°C in humidified air and 5% carbon dioxide. After incubation, the radioactivity of 100 µl of cell-free supernatant was counted with an automatic well-type gamma scintillation counter. The percent cytotoxicity was calculated from the formula:

\[
\text{% Activity} = \frac{\text{(experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})}{\text{(maximum } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})} \times 100
\]

Spontaneous ⁵¹Cr release was measured by incubating target cells with assay medium, and maximum release was measured by incubating target cells in water containing 5% sodium sulfate.

The values for spontaneous release were in the range of 5%–10% of the maximal release in all experiments. Maximal release was always greater than 5.0 × 10⁷ cpm·10⁴ cells.

CD4 was measured with Leu 3a antibody and CD8 with Leu 2a antibody (Becton Dickinson Monoclonal Center, Mountain View, CA, USA), using flow cytometry (Ortho Spectrum 3; Ortho Diagnostic Systems, Raritan, NJ, USA). The concentrations of IL-6 and IL-8 were determined by enzyme-linked immunosorbent assay (Toray-Fuji, Tokyo, Japan). Plasma concentrations of adrenaline and noradrenaline were measured by high-pressure liquid chromatography. Plasma cortisol was measured by radioimmunoassay.

Statistical comparisons were made by nonparametric methods with the Wilcoxon matched pairs signed ranks test for paired data, and the Mann-Whitney test for unpaired data. \( P < 0.05 \) was considered statistically significant. Values are presented as means ± SD.

**Results**

The three groups of patients were not different from each other in terms of age, body weight, duration of surgery, and volume of bleeding (Table 1).

Two hours after surgery and on postoperative day 1, the pain scores in groups M and MN were significantly lower than those in group C. On postoperative day 2, the pain scores dropped in each group, abolishing the differences between groups (Table 2). Postoperative analgesics such as indomethacin were administered to two patients in group MN, 3 patients in group M, and 3 patients in group C.

NK cell activity did not change significantly at 2 h after surgery in any group. However, the NK cell activity decreased on postoperative day 1 in groups MN (12.0 ± 2.7%) and M (25.4 ± 9.6%) compared with the respective baseline levels (38.3 ± 19.7%, 44.9 ± 13.2%).

**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Group C</th>
<th>Group M</th>
<th>Group MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 ± 7</td>
<td>45 ± 5</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55 ± 7</td>
<td>57 ± 6</td>
<td>55 ± 7</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>95 ± 16</td>
<td>102 ± 23</td>
<td>99 ± 20</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>247 ± 99</td>
<td>300 ± 99</td>
<td>272 ± 114</td>
</tr>
</tbody>
</table>

Values are means ± SD

There were no significant differences among groups

Group C, saline alone; group M, intrathecal morphine (0.5 mg) in saline; group MN, intrathecal morphine (0.5 mg) in saline with 5 µg noradrenaline