J Anesth (2004) 18:190–195
DOI 10.1007/s00540-004-0247-3

Journal of
Anesthesia

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

Tetsuya Yokota, Kiyoshi Uehara, and Yukiko Nomoto

Department of Anaesthesiology, Kanto Rohsai Hospital, 2035 Kizukisumiyoshi-cho, Nakahara-ku, Kawasaki 211-8510, Japan

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 Blood samples were withdrawn before and 2 h after surgery and on postoperative days 1, 2, and 7 to determine the NK cell activity, the ratio of T-helper/inducer cells (CD4) to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 L_{3-4} before induction of general

Address correspondence to: T. Yokota
Received: December 16, 2003 / Accepted: March 30, 2004

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(^{51}Cr) release assay, in which ^{51}Cr-labelled target cells (5×10^3) 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:

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

Spontaneous ^{51}Cr 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 5.0×10^3 cpm·10^{-4} 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

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

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

Table 2. Changes in pain scores

	2 h After surgery	POD 1	POD 2
Group C	3.5 ± 0.7	2.3 ± 0.5	1.2 ± 0.6
Group M	2.0 ± 0.6*	1.7 ± 0.6*	1.2 ± 0.5
Group MN	1.3 ± 0.5*	1.4 ± 0.5*	1.2 ± 0.5

* $P < 0.05$ compared with group C
Values are means ± SD
Two hours after surgery and on postoperative day (POD) 1, the scores in group M and group MN were significantly lower than those in group C

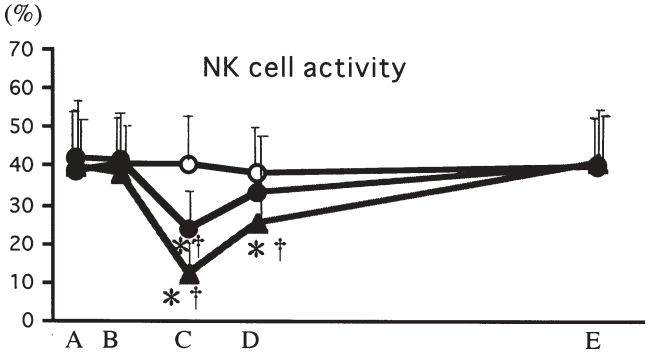

Fig. 1. Changes in natural killer (*NK*) cell activity expressed as percent cytotoxicity, at times *A* (before surgery), *B* (2 h after surgery), *C* (postoperative day [POD] 1), *D* (POD 2), and *E* (POD 7). The NK cell activity decreased on POD 1 in groups MN (12.0 ± 2.7%) and M (25.4 ± 9.6%) compared with their respective baseline levels (38.3 ± 19.7%, 44.9 ± 13.2%) before surgery. In group M, NK cell activity recovered to the baseline level on POD 2, whereas in group MN, NK cell activity on POD 2 (23.7 ± 8.0%) remained lower than the baseline level. *Open circles*, group C (saline alone); *closed circles*, group M (intrathecal morphine (0.5 mg) in saline; *closed triangles*, group MN (intrathecal morphine (0.5 mg) in saline with 5 μg noradrenaline. *$P < 0.05$ compared with baseline values; †$P < 0.05$ compared with group C

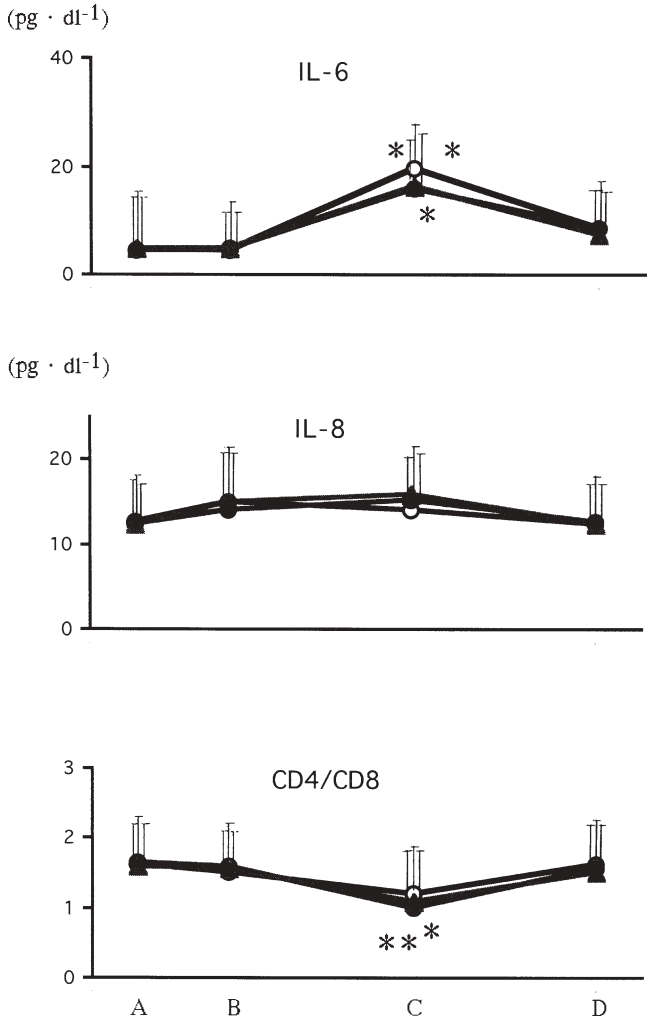

Fig. 2. Changes in plasma interleukin (*IL*)-6, IL-8, and CD4/CD8 at times *A* (before surgery), *B* (2 h after surgery), *C* (POD 1), and *D* (POD 2) in group C (*open circles*), group M (*closed circles*), and group MN (*closed triangles*). *POD*, postoperative day. *$P < 0.05$ compared with baseline values in each group

before surgery. In group M, NK cell activity recovered to the baseline level on postoperative day 2, whereas in group MN, NK cell activity on postoperative day 2 (23.7 ± 8.0%) remained lower than the baseline level. In group MN, the NK cell activity recovered to the baseline level on postoperative day 7 (Fig. 1).

The plasma IL-6 level increased on postoperative day 1 in all groups, but the IL-8 level showed no change in any group. There was no difference in the IL-6 levels on postoperative day 1 among the groups. The ratio of CD4/CD8 cells was significantly lower than the baseline level on postoperative day 1 in all groups. However, there was no difference in the ratio of CD4/CD8 among the groups (Fig. 2).

There were no changes in plasma adrenaline and noradrenaline concentrations in any group. Plasma cortisol concentration was significantly higher than baseline values 2 h after surgery and on postoperative day 1 and postoperative day 2 in all groups (Fig. 3).

Discussion

This study showed that intrathecal morphine suppressed NK cell activity, and that the addition of intrathecal noradrenaline had additional effects on NK cell activity.

There are several mechanisms by which opioids may alter human immunity.

First, opiates may induce immunosuppression via their effects on cytokines. Cytokines are important mediators of host defense mechanisms and the systemic

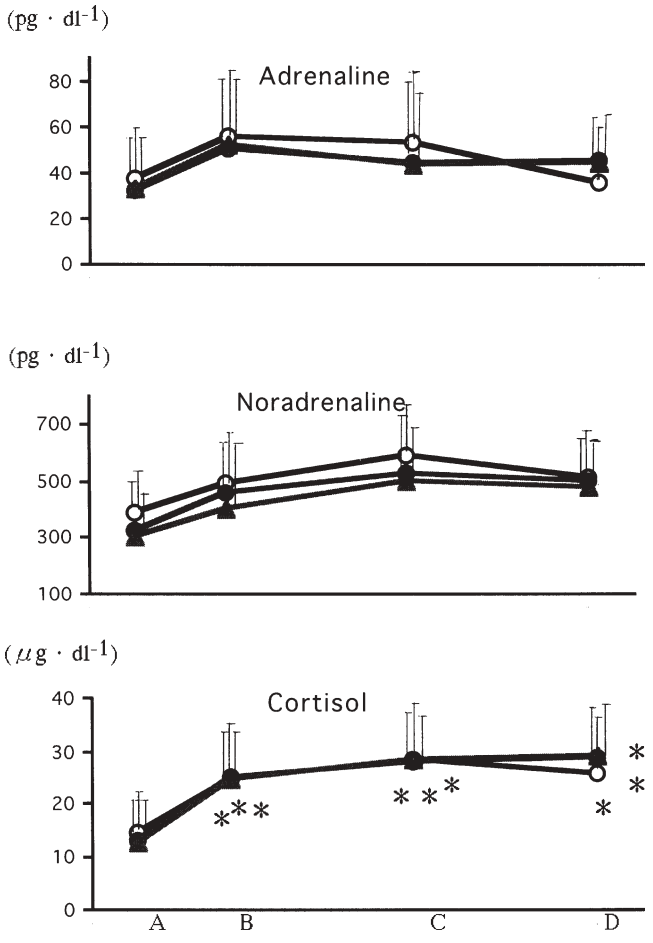

Fig. 3. Changes in plasma adrenaline, noradrenaline, and cortisol concentrations at times *A* (before surgery), *B* (2 h after surgery), *C* (POD 1), and *D* (POD 2) in group C (*open circles*), group M (*closed circles*), and group MN (*closed triangles*). There were no changes in plasma adrenaline and noradrenaline concentrations in any group. Plasma cortisol concentration was significantly higher than baseline values 2 h after surgery and on POD 1 and POD 2 in all groups. *POD*, postoperative day. $^{*}P < 0.05$ compared with baseline values in each group

inflammatory response. Changes in cytokine serum levels correlate with impaired cellular immune response, especially in patients undergoing major surgery. It was reported that in vitro incubation of human peripheral blood mononuclear cells with morphine resulted in suppressed production of interferon-γ and tumor necrosis factor-α [8]. In the present study, the plasma IL-6 level increased significantly on postoperative day 1 in all groups, however, the plasma IL-8 level did not change during the study. Several investigators have already reported that surgery and infection stimulate the production of a variety of endogenous mediators, especially IL-6 and IL-8. It seemed that the IL-8 level in the present study did not change because the operation entailed relatively little surgical invasion. Our results indicate that morphine does not induce NK cell activity suppression via its effect on cytokines, because there was no difference in IL-6 levels among the groups.

Second, opioids may also have an indirect effect on immune function through alterations in effector-cell populations. A decrease in effector-cell density in any immune compartment (thymus, peripheral blood, lymph node) will alter the results of functional assays that use mixed cell populations taken from any one compartment and tested without subset analysis of different cell types. Animal studies document sustained narcotic effects on effector-cell populations. For example, morphine pellet implantation in mice results in significant thymic and splenic atrophy, with an increased ratio of CD4/CD8 cells, but a decreased total number of both cell populations [9]. This effect was observed within 24 h after pellet implantation. The ratio of CD4/CD8 cells, a commonly used indicator of immunoregulatory cell imbalance, declined on postoperative day 1 in all groups in our study. However, our results showed that there were no differences in the ratios of CD4/CD8 cells among our groups.

Third, it has been suggested that, unlike exogenous opiates, endogenous opiate peptides enhance NK cell activity [10]. Opiate receptors have been demonstrated on various lymphocytes [11]; thus, exogenous opiate may compete with endogenous opiate peptides on this level, which could explain the net suppression of NK cell activity caused by large doses of opiates [10].

Finally, it is suggested that opioids can suppress immunity through effects on the central nervous system. For example, intraventricular administration of very small amounts of morphine (20–40 μg) results in depressed NK cell cytotoxicity similar to that observed after peripheral administration of much larger morphine doses (30–50 mg) [12]. This suggests a central nervous system-mediated effect after peripheral injection. Peripheral administration of N-methylmorphine, which does not cross the blood-brain barrier, has no immunosuppressive effect, whereas microinjections of N-methylmorphine into the third ventricle of the brain mediate immune suppression [13]. These findings suggest that brain opioid receptors are involved in the immunosuppressive effects of opioids, independent of direct drug effects on circulating lymphocytes.

Anesthesia, surgical stress, pain, and drugs used for analgesia have been reported to affect immune status, including NK cell activity [14]. Intrathecal morphine allows the use of anesthetics at low concentrations to maintain anesthesia [15]. Although the concentration of the anesthetics used for maintenance was lower in groups MN and M than in group C, there was no decrease in NK cell activity in group C. Surgical stress causes a postoperative rise in plasma cortisol and endogeneous catecholamine levels, leading to a de-

crease in NK cell activity [16]. In the present study, although plasma cortisol levels increased after surgery on postoperative days 1 and 2 in all groups, the NK cell activity did not decrease in group C. Pain also contributes to the immune response [17]. Drugs used for analgesia, such as indomethacin, have no effect on NK cell activity in the perioperative period [18]. However, different results were also reported [19]. In group C, there was no decrease in NK cell activity. This finding, taken together with our other results, indicated that anesthesia, surgical stress, pain, and drugs used for analgesia affected NK cell activity only a little in the present study.

An important mechanism of systemically administered opioids in causing analgesia is the activation of neurons in the midbrain and medulla with descending inhibitory projections to the spinal cord dorsal horn [20]. Chief among the inhibitory neurotransmitters released is norepinephrine, which diminishes substance P release from primary A and C afferents [21], and reduces the response of dorsal horn neurons to noxious stimulation. There are many studies supporting the relevance of descending spinal noradrenergic inhibition to the analgesia obtained from systemically administered opioids. However, there is little direct evidence of spinal noradrenaline release caused by systemically administered opioids in the nonanesthetized whole animal. Bouaziz et al. [22] indicated that intravenous morphine increased lumbar cerebrospinal fluid (CSF) concentrations of noradrenaline and acetylcholine in conscious sheep in a naloxone-reversible manner. Their microdialysis experiments suggested that these increases in noradrenaline and acetylcholine in CSF reflected the local release of these neurotransmitters from the spinal cord dorsal horn as a result of bulbospinal pathway activation. Gan et al. [23] demonstrated that noradrenaline-induced inhibition of NK cells was a function of multiple nonsequential blockades of the activation and maturation of NK cells. These blockades include the dysregulation of NK cell receptors, the blockade of NK-related cytokine secretion required for subsequent activation and maturation, and the interruption of recognition of target cells and of the lethal hit by the target-activated NK cells. Such multiple level inhibition by noradrenaline may reflect the inhibition of various signalling transduction pathways which are necessary for NK activation and function. In the present study, NK cell activity on postoperative day 2 remained lower than the baseline level in group MN. Plummer et al. [24] demonstrated that the antinociceptive effects of intrathecal morphine combined with noradrenaline were, to some degree, additive. In the present study, the pain scores in groups M and MN on postoperative day 1 were significantly lower than those in group C, but on postoperative day 2, the differences between the groups were abolished. These results suggest that postoperative analgesia was unaffected by the intrathecal administration of 5 μg of noradrenaline. The NK cell activity decreased on postoperative day 1 in groups MN and M. Although the NK cell activity recovered to the baseline level on postoperative day 2 in group M, it remained lower than the baseline level in group MN. Nelson et al. [25] investigated the time course of the immunologic and antinociceptive effects of morphine. Their results showed slight differences in the maximal immunological and antinociceptive effects of morphine; therefore, they suggested the posibility that different mechanisms may modulate the immunological and antinociceptive effects of morphine. It is desirable for the patients to have less postoperative pain while maintaining proper immune functions. Further study may be required to elucidate the influence of morphine and noradrenaline on immune functions.

In conclusion, our results indicated that the intrathecal administration of morphine caused a decrease in NK cell activity and that its combined use with noradrenaline prolonged the suppression of NK cell activity.

Acknowledgment. The authors gratefully acknowledge manuscript reviews by Professor Fumio Goto, Department of Anesthesiology, Gunma University.

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