Low-dose Cisplatin–5-Fluorouracil Prevents Postoperative Suppression of Natural Killer Cell Activity in Patients with Gastrointestinal Cancer

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Background: The effect of administering low-dose cisplatin (CDDP)–5-fluorouracil (5-FU) postoperatively on the activity of the immune system is not known. To clarify the effect on natural killer (NK) cell activity of treatment with low-dose CDDP–5-FU, we compared NK cell activity after surgery for gastrointestinal cancer in patients treated with low-dose CDDP–5-FU, a bolus dose of mitomycin C (MMC) or no anticancer drug.

Methods: Sixty-two patients consisted of three groups: low-dose CDDP–5-FU (n = 15), MMC (n = 20) and no-drug (n = 27). Chemotherapy was initiated immediately after surgery. NK cell activity was measured on the day before surgery (pre-op) and on postoperative days 7 (POD7) and 21 (POD21).

Results: The NK cell activities of the CDDP–5-FU group were 37.7% at pre-op, 36.1% on POD7 and 33.6% on POD21. However, the NK cell activities in the no-drug and MMC groups were significantly decreased on POD7 (from 36.6 to 24.8% and from 31.4 to 16.6%, respectively). The NK cell activity in the MMC group remained depressed on POD21 (18.6%) whereas that in the no-drug group recovered (31.6%).

Conclusions: Consecutive administration of low-dose CDDP–5-FU appears to be useful as postoperative adjuvant chemotherapy because of its preventive effect on NK cell suppression after surgery.

Key words: low-dose CDDP–5-FU – NK cell activity – gastrointestinal cancer – adjuvant chemotherapy

INTRODUCTION

The immediate postoperative period is appropriate for adjuvant chemotherapy (1), since treatment is most effective when there are fewer residual tumor cells (2). However, chemotherapy in addition to surgical stress may further impair host immunity because surgery causes various degrees of immunosuppression (3–7). Therefore, there is a need for postoperative chemotherapy which exhibits not only an essential anticancer effect but also a preservative effect on the host immune response.

There has been no establishment of a standard effective postoperative adjuvant chemotherapy for advanced gastric and colorectal cancer. The drug most commonly used in Japan is mitomycin C (MMC) (2); the maximum effective dose of MMC suppresses natural killer (NK) cell activity and pretreatment with this drug enhances the growth of the experimental metastases (8). Cisplatin (CDDP) and 5-fluorouracil (5-FU) are among the major agents which have been tested in clinical trials (2,9), and recently attention has turned to the combination therapy of low-dose CDDP–5-FU because of biochemical modulation (10,11). However, the effect of postoperative chemotherapy with a combination of low-dose CDDP–5-FU on the immune system has not been reported.

NK cells are important in host immunosurveillance (12) and NK cell activity is known as a good indicator of the host's resistance against tumor (13,14). A recent study demonstrated that the severity of the depression of NK cell activity depends on the severity of surgical stress (15).

To clarify the effect of treatment with low-dose CDDP–5-FU on NK cell activity, we compared NK cell activity after surgery for gastrointestinal cancer in patients treated with either low-dose CDDP–5-FU, bolus MMC or no anticancer drug.

MATERIALS AND METHODS

POSTOPERATIVE CHEMOTHERAPY

From June 1994 to December 1995, a total of 62 patients, 38 with gastric cancer and 24 with colorectal cancer, were included in this study. Informed consent for functional analysis of NK cells was obtained verbally from all patients when blood was first collected.
None of the patients received radiation, steroids or other therapy that influences the immune system. All patients who had received surgery with curative intent were included. The patients were retrospectively divided into three groups. Of the 35 patients given postoperative chemotherapy, one group (before December 1994) of 20 patients with locally advanced disease was treated with MMC (historical control) and the other group (after January 1995) of 15 similar patients was treated with CDDP-5-FU. The patients in the MMC group received a single intravenous injection of 8–12 mg/m² MMC on the day of surgery. The patients in the CDDP-5-FU group received a 1 h drip infusion of CDDP (5 mg/day) daily, along with a continuous 24 h infusion of 5-FU (500 mg/day) for 2 weeks from the day of surgery. Twenty-seven patients received no anticancer drug (no-drug group).

MEASUREMENT OF NK CELL ACTIVITY

Peripheral blood (10–15 ml) was drawn from each patient before breakfast and diluted twofold with phosphate-buffered saline (PBS) (Sigma Chemical, St Louis, MO). Specimens were overlaid on Ficoll-Conray gradients (specific gravity 1.077) (16) and centrifuged at 1350 r.p.m. for 30 min at room temperature. Mononuclear cells were collected as peripheral blood mononuclear cells (PBMC). After being washed three times with RPMI 1640 medium (GIBCO, Grand Island, NY), the PBMC were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (Flow Laboratories, VA) and the concentration was adjusted to 5 x 10⁶ cells/ml. Trypan blue dye exclusion was used to assess cell viability, which consistently exceeded 95%. PBMC were collected three times: on the day before surgery (pre-op) and again on postoperative days 7 (POD7) and 21 (POD21).

The erythroleukemia K562 cell line was used as a source of target cells. They were cultured in RPMI 1640 with 10% FCS. They were labeled with sodium chromate (Na²⁵¹CrO₄, 3.7 MBq for 1 x 10⁶ cells) in culture media at 37°C in air with 5% CO₂ for 1 h. Following incubation, the cells were washed three times in RPMI 1640 and adjusted to 1 x 10⁶/ml with RPMI 1640 containing 10% FCS.

NK cell activity was measured with the standard 4 h ⁵¹Cr-release assay (17). Each 100 μl of PBMCs (effector) and K562 cells (target) were combined at an effector-to-target (E:T) ratio of 50:1 in 96-well microtiter plates. Maximum release was determined by the addition of detergent to K562 cells. Spontaneous release was measured by culturing K562 cells without PBMC. After a 4 h incubation at 37°C in air with 5% CO₂, supernatants were harvested and radioactivity was assessed using a gamma counter (Alfa, Japan). All assays were performed in triplicate and the value was calculated as the mean of triplicate cultures. The percentage cytotoxicity was determined as (experimental c.p.m. – spontaneous c.p.m.)/(maximum c.p.m. – spontaneous c.p.m.) x 100. The values of spontaneous release were less than 10% of the maximum release in all experiments. Because of the limitation of the amount of samples, only the measurement at an E/T ratio of 50:1 was performed in this experiment.

STATISTICAL ANALYSIS

Data are reported as mean ± standard error (SE). Analysis utilized the chi-squared test, one-way repeated measures analysis of variance (ANOVA) and one-way factorial ANOVA and Scheffe’s test as a post-hoc test when results of the above tests were significant. A level of $P < 0.05$ was considered to be statistically significant.

RESULTS

Patient characteristics are shown in Table 1. All patients had undergone curative resection. There were no significant differences among the three groups, especially with regard to the operation performed, the length of surgery and volume of blood loss, which were the criteria for estimating surgical stress. There were also no differences among the three groups in the complications experienced during the study, anesthesia, management of postoperative pain or blood transfusion, each of which may affect host immune function (data not shown).

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>CDDP-5-FU</th>
<th>MMC</th>
<th>No-drug</th>
<th>$P$ value</th>
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<tbody>
<tr>
<td>(n = 15)</td>
<td>(n = 20)</td>
<td>(n = 27)</td>
<td></td>
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</tr>
<tr>
<td>Age (years)</td>
<td>64 ± 2</td>
<td>63 ± 3</td>
<td>67 ± 2</td>
<td>NS</td>
</tr>
<tr>
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<td>10:10</td>
<td>16:11</td>
<td>NS</td>
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<tr>
<td>Disease and operation</td>
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<tr>
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<td>6</td>
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<td>276 ± 11</td>
<td>278 ± 13</td>
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<td>Blood loss (ml)</td>
<td>686 ± 123</td>
<td>498 ± 109</td>
<td>589 ± 140</td>
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</table>

Values are expressed as mean ± SE. NS: not significant.
The changes in mean NK cell activity are shown in Fig. 1. Preoperative NK cell activity in the three groups did not differ significantly (36.6 ± 3.0, 31.4 ± 2.7 and 37.7 ± 4.3 in the no-drug, MMC and CDDP-5-FU groups, respectively). The change in NK cell activity in the three groups differed according to the treatment administered after surgery. The mean NK cell activity in the no-drug group was significantly depressed on POD7 (24.8 ± 2.8%) and returned to the preoperative value on POD21 (31.6 ± 2.9%). In the MMC group, the mean NK cell activity was significantly decreased on POD7 (16.6 ± 2.2%) and did not recover on POD21 (18.6 ± 2.5%). The mean NK cell activity in the CDDP-5-FU group showed no significant decrease on POD7 and POD21 (pre-op, 37.7 ± 4.3%; POD7, 36.1 ± 4.3%; POD21, 33.6 ± 3.4%). On POD7, NK cell activity in the no-drug group and in the MMC group was significantly lower than that in the CDDP-5-FU group. On POD21, NK cell activity in the MMC group was significantly lower than that in both the no-drug group and the CDDP-5-FU group.

Table 2 shows the type and severity of adverse event associated with the anticancer drugs used in this study (18). No significant drug toxicity was exhibited by either group.

The present study showed that low-dose CDDP-5-FU prevented the suppression of postoperative NK cell activity, while the bolus administration of MMC impaired the recovery of the depressed NK cell activity. As reported in other studies (3,4,7), impairment of NK cell activity was demonstrable on POD7 and preoperative levels of NK cell activity were reattained by POD21. The transient depression of NK cell activity observed in the no-drug group on POD7 was probably due to surgical stress. The decreased NK cell activity in this group returned to its preoperative value on POD21 as the patients recovered from surgery (Fig. 1). According to these findings, we have regarded POD7 as the time point which was affected by surgical stress and POD21 as that recovered from surgical stress. Despite the absence of randomization, there were no significant differences in preoperative NK cell activity. The characteristics of the patients within each of the three groups were similar (Table 1). Moreover, except for anticancer drugs administered there were no significant differences with regard to such immunomodulatory factors as postoperative complications, anesthesia, management of postoperative pain and blood transfusion (data not shown). Consequently, the changes in NK cell activity observed in this study were thought to reflect the effects of the anticancer drugs in addition to surgical stress.

**DISCUSSION**

The present study showed that low-dose CDDP-5-FU prevented the suppression of postoperative NK cell activity, while the bolus administration of MMC impaired the recovery of the depressed NK cell activity. As reported in other studies (3,4,7), impairment of NK cell activity was demonstrable on POD7 and preoperative levels of NK cell activity were reattained by POD21. The transient depression of NK cell activity observed in the no-drug group on POD7 was probably due to surgical stress. The decreased NK cell activity in this group returned to its preoperative value on POD21 as the patients recovered from surgery (Fig. 1). According to these findings, we have regarded POD7 as the time point which was affected by surgical stress and POD21 as that recovered from surgical stress. Despite the absence of randomization, there were no significant differences in preoperative NK cell activity. The characteristics of the patients within each of the three groups were similar (Table 1). Moreover, except for anticancer drugs administered there were no significant differences with regard to such immunomodulatory factors as postoperative complications, anesthesia, management of postoperative pain and blood transfusion (data not shown). Consequently, the changes in NK cell activity observed in this study were thought to reflect the effects of the anticancer drugs in addition to surgical stress.
Little information is available on the immunological effects of low-dose CDDP-5-FU or the synergistic effects of such drug therapy. However, there are some reports concerning the effect of CDDP on the host immune system, optimum low-dose CDDP augmented NK cell activity in vitro and in vivo (19–21). Other studies suggest that immune suppressor cells and circulating suppressive factors are produced during surgery and depress the host's immune function (3,5,6). CDDP can abrogate these suppressor cells (22,23). On the other hand, 5-FU-based chemotherapy is generally considered to be associated with immunosuppression (24,25) and it appears that 5-FU does not augment NK cell activity. Therefore, we presume that in treatment with CDDP–5-FU, the CDDP rather than the 5-FU activates NK cells and/or eliminates suppressor cells and, accordingly, preserves the NK cell activity postoperatively. The mechanism of the effects of CDDP–5-FU on other immune parameters requires further study. By contrast, the MMC bolus injection suppressed NK cell activity continuously up to POD21, while the suppression of NK cell activity in the no-drug group recovered by POD21 (Fig. 1). This result in the MMC group may be due to the combined effects of surgical stress and drug-induced bone marrow suppression. Lundy et al. (26) reported that perioperative immunosuppression could promote the growth of micrometastases. Saijo et al. (8) demonstrated that the suppression of NK cell activity by MMC enhanced the growth of experimental pulmonary and liver metastases in mice. Our results suggest that MMC given by bolus injection may be contraindicated as postoperative adjuvant chemotherapy. However, there are some reports concerning the effect of MMC treatment, even in the immediate postoperative period (27,28). MMC treatment, even immediately after surgery.

References
