# EFFECTS OF PERIOPERATIVE CIMETIDINE ADMINISTRATION ON NATURAL KILLER CELLS IN PATIENTS WITH GASTROINTESTINAL CANCER

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**Abstract**

**Objective: To study the effects of perioperative use of cimetidine on natural killer (NK) cells in gastrointestinal (GI) cancer patients. Methods: 49 GI cancer patients were randomized into treatment group which took cimetidine in the perioperative period, and control group which did not take the drug. NK cells were measured by immunocytochemical method, using mouse-anti-human $CD_{57}$ monoclonal antibody as the primary antibody. Blood samples from 20 healthy volunteers were treated in the same way as normal control. Comparisons were made within and between groups. Results: The NK cell percentage of normal control was 18.50±2.31. Both groups of patients had significantly lower than normal NK percentages before treatment ($P<0.05$). NK cell percentages at admission, before operation, on the 2nd and the 10th postoperative days were 14.60±3.91, 15.64±3.61, 17.40±3.28, 20.68± 4.13, respectively, for the treatment group, and 14.88± 2.76, 13.17±2.93, 14.50±2.77, 15.67±2.55, respectively, for control group. The difference between the two groups was statistically significant. Conclusion: Perioperative cimetidine application can help restore NK cells. The drug may be useful to reverse postoperative immuno-depression in GI cancer patients.**

**Key words: Cimetidine, Natural killer cells, Gasstrointestinal cancer.**

Cimetidine (CIM) is a histamine type 2 receptor antagonist widely used to treat peptic ulcers. It also has important effects on the immune system. The administration of CIM has been found to preserve, to some degree, patient's perioperative immune capability.[1] However, whether the use of CIM can promote NK cells in patients with gastrointestinal (GI) cancer is still open to question. To investigate this issue, we carried out the following study.

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## MATERIALS AND METHODS

### The Study Design

From September 1997 to May 1998, 125 consecutive patients with GI cancers entered the Department of Oncology, the 2nd Affiliated Hospital of Hubei Medical University. The criteria for entry into this study were: (1) primary GI cancers fit for surgery; (2) no preoperative evidence of distant metastasis; (3) no previous history of immune-impairing chronic diseases such as diabetes mellitus; (4) no history of preoperative chemotherapy, radiotherapy or immunotherapy. 49 eligible patients were recruited into this study. After giving informed consent, these patients were randomized into treatment and control groups. The former began oral CIM (Tagamet, Tianjin Smith Kline & French Laboratories Ltd.) intake at the dose of 400 mg, tid, 7d before operation. During and after operation, CIM 600 mg was given iv drip, bid, until the 10th postoperative day. Patients in the control group only received their routine treatment. All the patients in both groups underwent curative resection for cancer.

### Separation of Peripheral Blood Lymphocytes (PBL) and Immunocytochemical Stain

From all patients, 2 ml of venous blood was taken and heparinized at admission, before operation, on the 2nd and the 10th postoperative days, respectively. PBL obtained by standard Ficoll-Hypague gradient

centrifugation method were smeared on slides, dried and fixed for immunocytochemical stain according to the method by Coca et al.[2] The primary antibody was mouse-anti-human $CD_{57}$ monoclonal antibody (Sigma, USA). Primary antibody was visualized with repavidin-biotin-peroxidase supersensitive kit (Wuhan Boster Bioengineering Co. Ltd, China). PBL from 20 healthy volunteers were processed in the same way as normal controls.

The slides were viewed under binocular microscope (Olympus, Japan) by an independent viewer. $CD_{57}^+$ cells were counted in 200 lymphocytes as NK cells. NK cells percentages were calculated and expressed as mean±standard deviation ($x\pm s$).

### Statistical Analysis

Analysis of variance (ANOVA) was employed to process the data within group. Student's $t$ test was used to compare the difference between groups. The tests were two-tailed with the level of significance $P$=0.05.

## RESULTS

### The Clinicopathological Features of the Patients

As shown in Table 1, there was no statistically significant differences in the variables between the two groups ($P$>0.05).

### Percentages of NK Cells at Different Times in the Perioperative Period

From Table 2, we can see that patients in both groups had significantly lower NK cell percentages than normal control before treatment (14.6±3.91, 14.88±2.76 versus 18.50±2.31, $P$<0.05, $t$ test). This is one piece of evidence suggesting these patients had decreased cellular immunity.

In the control group, NK cell percentage continued to fall as the disease progressed (from 14.88±2.76 on admission to 13.17±2.93 before operation, $P$<0.05, ANOVA). This downward trend was curbed and even slightly reversed by curative operation (13.17±2.93 before operation versus 14.50± 2.77 on the 2nd postoperative day, $P$>0.05, ANOVA), although the difference was statistically not significant. However, on the 10th postoperative day, NK cell percentage had increased significantly and surpassed the level on admission. Nevertheless, it was still below the normal control level. These findings demonstrate that curative surgery can remove the immunity-impairing action of tumor burden, but the effect is not enough.

*Table 1. The clinicopathological features of 49 patients in the study*

| Item | Treatment | Control |
|---|---|---|
| Age (yr.) | | |
| Mean (range) | 50(25–73) | 53(27–28) |
| Gender | | |
| Male | 13 | 16 |
| Female | 12 | 8 |
| Tumor sites (No) | | |
| Stomach | 6 | 5 |
| Colon | 3 | 3 |
| Rectum | 16 | 16 |
| Pathological types | | |
| Tubular adenocarcinoma | 14 | 12 |
| Papillary adenocarcinoma | 3 | 3 |
| Villous adenocarcinoma | 2 | 1 |
| Signet-ring-cell carcinoma | 2 | 3 |
| Mucous adenocarcinoma | 4 | 5 |
| TNM stages (No) | | |
| I | 3 | 5 |
| II | 7 | 9 |
| III | 9 | 6 |
| IV | 6 | 4 |
| Differentiation (No) | | |
| Well differentiated | 5 | 6 |
| Moderately differentiated | 8 | 7 |
| Poorly differentiated | 12 | 11 |

*Table 2. NK cell percentages in the perioperative period in this study ($x\pm s$)*

| Item | No. | NK % | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Normal control | 20 | 18.50±2.31 | | | |
| Treatment group | 25 | 14.60±3.91* | 15.64±3.61* | 17.40±3.28 | 2.068±4.13* |
| Control group | 24 | 14.88±2.76* | 13.17±2.93** | 14.50±2.77** | 15.67±2.55** |

A: NK% before treatment; B: NK% before operation; C: NK% on the 2nd postoperative day; D: NK% on the 10th postoperative day. *$P$<0.05 comparison between the values of both groups and the value of normal control; **$P$<0.01 comparison in the same time period between treatment and control groups. Student's $t$ test.

In the treatment group, CIM seemed to stimulate NK cells. 7d after CIM application, the NK cell

percentage ceased decreasing and even increased slightly, although the difference was not statistically significant (14.60±3.91 on admission versus 15.64± 3.61 before operation, $P>0.05$, ANOVA). On the 2nd postoperative day, it rose well above the preoperative level (17.40±3.28 versus 15.64±3.61, $P<0.01$, ANOVA). Thereafter, the upward trend continued. On the 10th postoperative day, the value even exceeded that of the normal control group (20.68±4.13 versus 18.50±2.31, $P<0.05$, $t$ test).

## DISCUSSION

NK cells are a group of lymphocyte subpopulations with unique characteristics. They are actively involved in host's immune surveillance against tumor cells. These cells are special in that they can, without mediators, recognize and destroy tumor cells by their cytotoxic activity.[3] NK cells are especially active at the sites of election for metastasis formation.[4] The degree of intratumoral infiltration of NK cells has been found to be positively associated with prognosis.[2]

To date, surgical resection has been the only method that can offer possible cure for gastrointestinal cancers. However, operation itself is a double-edged sword to cancer patients in terms of tumor immunology. On the one hand, operation removes tumor burden which is immune suppressive. This will help bring about improved clinical status of the patients. On the other hand, the operation itself is a major blow to the immune system. There is much evidence to suggest that surgical patients undergo a period of transient cellular immunodepression immediately after surgery. Earlier studies already demonstrated that T helper cells decreased and T suppressor cells increased significantly as soon as one day after surgery.[5] Following this report appeared many subsequent studies, which confirmed the findings. Recent researches have lent further support to the concept. These studies revealed decreased NK cytotoxicity against tumor target cells immediately after surgical resection of rectal cancer.[6,7] All these studies have confirmed that major surgery induces a temporary postoperative immunodepression. For cancer patients, one of the most important functions adversely affected by this immunodepression would be the antitumor response itself. This immunodepression might enhance the growth of residual tumor or micrometastasis already present at the time of surgical removal. Therefore, how to effectively improve the perioperative cellular immunity of cancer patients remains a major issue of very important clinical significance.

Many researches have been carried out to solve this problem. For example, a number of cytokines are used in clinical trials. These include recombinant human interferon-α (γ-HuIFN-α) alone,[8] recombinant interleukin 2 (rIL-2) alone, or rIL-2 plus IFN-γ.[6,9] While these agents have varied degrees of activity to boost NK cells, their adverse side effects and costs are also great concerns.

Besides biological agents, some small molecule drugs such as levamisole and CIM are also well known immunomodulators. Studies have demonstrated that CIM can promote NK function in ovarian cancer,[10,11] chronic lymphocytic leukemia,[12] and nasopharyngeal cancer.[13] In the current study, we found that perioperative administration of CIM can also promote the recovery of NK cells in GI cancer patients. For patients who took this drug, the recovery of NK cells after operation was quick and profound NK cell percentage was significantly higher on the 2nd postoperative day than before surgery. On the 10th postoperative day, it had already reached normal level. However, for patients who did not take the drug, NK cell recovery was much slower and less obvious.

In summary, our current study has demonstrated that perioperative CIM administration to GI cancer patients helps restore NK cells. This has potential therapeutic application. Since NK cells represent at least one of the mechanisms of destroying circulating tumor cells and inhibiting metastasis, to increase these cells in the perioperative period with this low cost, convenient and almost nontoxic immune modulator might be a valuable and practical measure to boost postoperative cellular immunity in GI cancer patients. As to the question whether this treatment modality can help decrease recurrence or increase survival. We are now closely following our patients and further studies are under way.

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