CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

CIMETIDINE REDUCES IMPAIRMENT OF CELLULAR IMMUNITY AFTER CARDIAC OPERATIONS WITH CARDIOPULMONARY BYPASS

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Objective: Depressive effects of cardiopulmonary bypass on cell-mediated immune responses may lead to postoperative infectious complications. We previously reported that cimetidine reduced postbypass depression of the cytotoxic activity of natural killer cells. This study evaluated cimetidine as an agent to preserve cellular immunity after cardiac operations. **Methods:** In a prospective randomized study, 20 patients were divided into two groups of equal size. Cimetidine-group patients received 400 mg of cimetidine intravenously before bypass and a 33 mg/hr intravenous infusion of cimetidine after the operation, continuing until the fifth postoperative day. Control-group patients received conventional perioperative therapy. Lymphocyte subsets, natural killer cell activity, percentage of CD56⁺CD16⁺ (percentage of natural killer cells), and percentage of CD11b⁺CD8⁺ (percentage of suppressor T lymphocytes) were measured perioperatively. **Results:** Although temporary postoperative reductions in percentages of CD3⁺, CD4⁺, and CD56⁺CD16⁺ cells were observed in both groups, CD8⁺ percentages on postoperative day 1 and CD11b⁺CD8⁺ percentages on postoperative days 1 and 3 in the cimetidine group were significantly lower compared with those in the control group ($p = 0.01$, $p = 0.004$, and $p = 0.02$, respectively). Temporary postoperative reduction of natural killer cell activity was also observed in both groups, but the natural killer cell activity on postoperative day 1 in the cimetidine group (17.1%) was significantly higher ($p = 0.02$) than that in the control group (8.20%). **Conclusions:** Cimetidine counteracts depressive effects of cardiopulmonary bypass on cell-mediated immunity and may possibly reduce postoperative susceptibility to infection. (J Thorac Cardiovasc Surg 1998;116:312-8)

Patients undergoing cardiac operations with cardiopulmonary bypass (CPB) are subjected to many kinds of postoperative insults to cellular immunity. CD4⁺ (helper/inducer) lymphocyte reduction,¹⁻⁹ CD8⁺ (suppressor/cytotoxic) lymphocyte elevation,¹⁻⁵ and reduction in natural killer (NK) cell cytotoxic activity^{3,4,6} are seen on postoperative days

(PODs) 1 to 3, and for almost all of these, levels will return to normal by POD 7. CD4⁺ (helper T) cells can help B cells make antibodies and activate the actions of other effector cells. On the other hand, CD8⁺ (suppressor T) cells reduce the immune responses of other T cells or B cells. NK cells can activate functions of macrophages. Decreased numbers of B lymphocytes^{5,10} and poor response to mitogens^{2,4,7,8} have also been reported. These depressive effects of CPB on cellular immunity increase the risk of infectious complications such as septic multiorgan failure.^{1,2}

Cimetidine, a histamine type 2 receptor antagonist, has been shown to increase immunoreactivity by activating interleukin-2 production,^{11,12} inhibiting suppressor T lymphocyte activity,^{11,13,14} and enhancing NK cell activity.¹⁵ Improved immunoreactivity resulting from administration of cimetidine

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has been considered to be associated with prolonged survival time in patients with malignant diseases¹⁶⁻¹⁹ and improvement in resistance to infection.²⁰ On the basis of these clinical studies, many physicians have suggested the possibility of using cimetidine as an agent of adoptive immunotherapy. We previously reported that the perioperative use of cimetidine preserved NK cell activity after cardiac operations with CPB.²¹ The objective of this study was to investigate the effect of cimetidine on cellular immunity after CPB in more detail.

Patients and methods

Twenty patients undergoing either coronary artery bypass grafting or heart valve operations, without evidence of concomitant malignancy or immunologic disorder, were included in this study. The study was approved by the ethics review board of Yamanashi Central Hospital, Yamanashi, Japan, and informed consent was obtained from each patient. In this prospective randomized trial, the patients were divided into two groups of equal size. Patient characteristics are summarized in Table I. All patients received high-dose fentanyl (50 µg/kg) anesthesia to which morphine (0.4 mg/kg) and midazolam were added. The cimetidine group of patients received an infusion of 400 mg of cimetidine (SmithKline Beecham, Tokyo, Japan) intravenously over 20 minutes soon after the induction of anesthesia and before CPB. In addition, patients in the cimetidine group were given a 33 mg/hr continuous intravenous infusion of cimetidine (normal daily dose for antacid therapy) starting soon after the operation and continuing until POD 5, when generally suppressed cellular immunity returns to an almost normal level. The control group of patients received conventional perioperative therapy.

Extracorporeal perfusion was performed with use of moderate hypothermia with a Sarns 7400MDX roller pump, a Sarns Turbo 9443 membrane oxygenator, and a prime of 1.5 L of standard electrolyte solution containing sodium bicarbonate, mannitol, dextran, and heparin. All patients received cefazolin sodium as antibiotic prophylaxis during (2 gm) and after (4 gm/day for 5 days) the operation. Autologous blood transfusions were administered if the patient tolerated blood storage before the operation. Patients who required homologous transfusion were excluded from the study.

Immunologic investigations. Peripheral blood samples were taken preoperatively and on PODs 1, 3, and 7. Peripheral blood mononuclear cells were prepared soon after the sampling.

Flow cytometric analysis. Lymphocytes were isolated with a Ficoll-Hypaque gradient centrifugation (Ficoll, Pharmacia, Piscataway, N.J.; Hypaque sodium diatrizoate, Winthrop Laboratories, New York, N.Y.) at 1500 rpm at 20° C and were washed and suspended in a balanced salt solution. Lymphocytes were analyzed after staining with fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies. The following monoclonal antibodies were used to detect lymphocyte subpopulations

Table I. Patient characteristics and surgical data

	Cimetidine group (N = 10)	Control group (N = 10)	p Value
Age (yr)	62.0 ± 2.5	62.6 ± 3.6	0.89
Weight (kg)	58.1 ± 2.6	58.7 ± 1.7	0.90
Sex			
Male	8	7	
Female	2	3	
CABG	7	6	
Valve operation	3	4	
Autologous blood transfused (ml)	480 ± 112	440 ± 122	0.88
CPB duration (min)	87.7 ± 8.6	87.5 ± 7.1	0.99
ACC duration (min)	48.0 ± 6.3	46.8 ± 4.8	0.88
Lowest rectal temperature during CPB (°C)	29.1 ± 0.29	28.9 ± 0.34	0.64

Values are given as the mean plus or minus the standard error of the mean. CABG, Coronary artery bypass grafting; ACC, aortic crossclamping.

(Becton Dickinson, Mountain View, Calif.): CD3 (mature T cell), CD4 (helper/inducer T cell), and CD8 (suppressor/cytotoxic T cell). The coexpressions of the CD11b marker on CD8 cells and the CD56 marker on CD16 cells were studied with the use of two-color immunofluorescence to detect suppressor T cells and NK cells, respectively. The cells stained with antibodies were counted with a flow cytometer (FACStar, Becton Dickinson).

NK cell cytotoxicity assay. Lymphocytes isolated with Ficoll-Hypaque gradient (effector cells) and chromium 51-labeled K-562 cells (target cells) were mixed in an RPMI 1640 medium at an effector to target ratio of 20:1. After incubation in 5% carbon dioxide for 4 hours at 37° C, NK cell cytotoxicity was calculated on the basis of the radioactivity of chromium 51, which was estimated with use of a scintillation counter. NK cell activity was expressed as the percentage of cytolysis according to the following expression: percent cytolysis = (cpm experimental release – cpm spontaneous release)/(cpm maximum release – cpm spontaneous release) × 100, where cpm is counts per minutes. The spontaneous release was the amount of radioactivity released from target cells in the growth medium and the maximum release was the total radioreactivity in the target cells.

Statistical analysis. Results are presented as the mean plus or minus the standard error of the mean. Two-way analysis of variance with adjustment for repeated measures was used for intergroup comparison. The calculated significant probabilities were adjusted by Dunnett's multicomparison method to control type I errors. Analysis of variance with the Bonferroni/Dunn multicomparison method as a post hoc test was used for intragroup comparison. Comparisons of the patient characteristics and surgical data were made with the Student's *t* test. All data were computed by the MIXED procedure in SAS software release 6.12.

Table II. Changes in perioperative immunologic data

	Group	Preop.	POD 1	POD 3	POD 7
Lymphocyte count (per mm ³)	Cimetidine	1782 ± 181	806 ± 84.9 (0.0006)	1476 ± 183 (0.26)	1997 ± 297 (0.43)
	Control	1935 ± 148	822 ± 71.2 (<0.0001)	1625 ± 200 (0.26)	2034 ± 260 (0.72)
CD3 (%)	Cimetidine	62.2 ± 1.37	31.8 ± 3.33 (<0.0001)	55.5 ± 4.33 (0.09)	66.5 ± 2.14 (0.28)
	Control	64.2 ± 1.59	33.3 ± 2.51 (<0.0001)	59.3 ± 3.34 (0.22)	64.3 ± 2.50 (0.98)
CD4 (%)	Cimetidine	41.9 ± 1.80	21.0 ± 2.14 (<0.0001)	41.9 ± 1.92 (0.99)	51.0 ± 2.65 (0.01)
	Control	44.8 ± 2.82	19.3 ± 2.23 (<0.0001)	37.4 ± 3.18 (0.04)	48.0 ± 2.83 (0.37)
CD8 (%)	Cimetidine	24.7 ± 1.46	19.5 ± 1.79 (0.07)*	8.20 ± 1.90 (0.03)	22.5 ± 2.54 (0.44)
	Control	23.2 ± 2.28	28.2 ± 2.63 (0.09)*	23.3 ± 1.79 (0.98)	22.2 ± 1.64 (0.73)
CD4/CD8 ratio	Cimetidine	1.76 ± 0.15	1.14 ± 0.13 (0.06)†	2.50 ± 0.24 (0.03)‡	2.60 ± 0.38 (0.01)
	Control	2.15 ± 0.27	0.76 ± 0.12 (<0.0001)†	1.67 ± 0.17 (0.15)‡	2.31 ± 0.26 (0.61)
CD11b ⁺ CD8 ⁺ (%)	Cimetidine	10.8 ± 1.41	7.40 ± 1.05 (0.10)§	5.60 ± 0.59 (0.01)	10.3 ± 0.67 (0.78)
	Control	9.40 ± 1.78	15.7 ± 2.80 (0.003)§	9.70 ± 1.15 (0.88)	9.52 ± 0.52 (0.95)
CD11b ⁺ CD8 ⁺ cell count (per mm ³)	Cimetidine	186 ± 32.9	57.4 ± 8.42 (0.001)¶	80.6 ± 12.4 (0.007)#	210 ± 40.8 (0.53)
	Control	175 ± 28.8	131 ± 28.6 (0.26)¶	157 ± 24.5 (0.65)#	192 ± 24.2 (0.66)
CD56 ⁺ CD16 ⁺ (%)	Cimetidine	14.4 ± 2.08	27.5 ± 4.22 (0.0001)	7.77 ± 0.49 (0.03)	7.60 ± 1.32 (0.03)
	Control	14.5 ± 2.35	19.2 ± 2.01 (0.12)	6.88 ± 0.94 (0.01)	8.22 ± 1.08 (0.04)
CD56 ⁺ CD16 ⁺ cell count (per mm ³)	Cimetidine	254 ± 52.5	207 ± 28.6 (0.37)	115 ± 7.10 (0.01)	159 ± 41.5 (0.07)
	Control	284 ± 52.7	161 ± 23.0 (0.02)	111 ± 21.6 (0.001)	173 ± 38.6 (0.04)
NK cell activity (%)	Cimetidine	36.8 ± 3.58	17.1 ± 1.50 (<0.0001)**	19.3 ± 2.11 (0.0005)	36.7 ± 3.34 (0.98)
	Control	37.8 ± 5.99	8.20 ± 2.03 (<0.0001)**	15.9 ± 1.99 (<0.0001)	34.3 ± 3.96 (0.46)

Values are given as the mean plus or minus the standard error of the mean. Data of the lymphocyte subsets are given as the percentage of the peripheral blood mononuclear cells. *p* Values in parentheses are for comparisons with preoperative data. All statistically significant values with intergroup comparisons are footnoted.

**p* = 0.01.

†*p* = 0.04.

‡*p* = 0.03.

§*p* = 0.004.

||*p* = 0.02.

¶*p* = 0.02.

#*p* = 0.02.

***p* = 0.02.

Results

Table I shows the clinical characteristics of the patients. There were no significant differences in age, sex ratio, or CPB time between the two groups. Autologous blood transfusions were performed in seven patients in the cimetidine group and six patients in the control group; there were no significant differences in the average amount of autologous blood transfused between the groups (*p* = 0.87). None of the patients died during hospitalization and none had any significant postoperative complications, with the exception of one. One case of postoperative infection was observed during this study, which occurred in a 63-year-old female patient in the control group who underwent coronary artery bypass grafting. Her early postoperative course was uneventful, but a fever developed on POD 5. Because a large quantity of pus was discharged from the mediastinal wound on POD 7, emergency reexploration was performed. It became apparent that there was extensive infection throughout the pericardial

space. After irrigation with a large amount of saline solution and débridement of the necrotic tissue, the chest was closed with three drainage tubes without sternal closure. A culture of the discharge revealed methicillin-resistant *Staphylococcus aureus*. The mediastinitis resolved after 3 weeks of continuous saline solution irrigation via the tubes and administration of intravenous vancomycin. It is noteworthy that the NK cell activity in this patient both before the operation and on POD 1 was extremely low (3% and 2%, respectively).

Lymphocyte subsets. The perioperative changes of lymphocyte subsets are shown in Table II. A temporary postoperative reduction of CD8 level was observed in the cimetidine group. There was a significant difference in the CD8 level on POD 1 between the groups (*p* = 0.01): the cimetidine group had a 21.2% reduction whereas the control group had a 21.7% elevation, in comparison with the preoperative levels. The CD4/CD8 ratio on POD 1 was decreased in both groups compared with the preoperative level. There were significant differ-

Fig. 1. Changes in (a) the percentage of suppressor T lymphocytes (CD11b⁺CD8⁺ cell) in peripheral blood and (b) their absolute count before and after CPB. Asterisks and pound signs show values that were significantly different in intergroup comparison. * $p = 0.004$; # $p = 0.02$; ** $p = 0.02$; ## $p = 0.02$.

ences in the ratio between the groups on POD 1 ($p = 0.04$) and POD 3 ($p = 0.03$).

Two-color analysis. In the control group, the percentage of CD11b⁺CD8⁺ lymphocytes (suppressor T lymphocytes) on POD 1 was significantly elevated compared with the preoperative level ($p = 0.003$). The percentage in the cimetidine group was significantly decreased on POD 1 ($p = 0.04$) and POD 3 ($p = 0.01$) compared with the preoperative level. Intergroup comparisons showed significant differences in the percentage of CD11b⁺CD8⁺ lymphocytes on POD 1 ($p = 0.004$) and POD 3 ($p = 0.02$) (Fig. 1, a).

The percentages of CD56⁺CD16⁺ lymphocytes (NK cells) were elevated on POD 1 (cimetidine group, $p = 0.0001$; control group, not significant) and then significantly reduced in the period between POD 3 and POD 7 in both groups. There were no significant differences in the percentages of CD56⁺CD16⁺ lymphocytes between the groups (Fig. 2, a).

NK cell cytotoxicity assay. A temporary, postoperative reduction of the NK cell activity was observed in both groups. The NK cell activity on POD 1 in the cimetidine group ($17.1\% \pm 1.50\%$) was significantly higher ($p = 0.02$) than that in the control group ($8.20\% \pm 2.03\%$) (Fig. 3).

Discussion

This clinical study has demonstrated that cell-mediated immunity is impaired after cardiac opera-

tions involving CPB: CD3⁺ cell count, CD4⁺ cell count, CD56⁺CD16⁺ cell count, and NK cell activity were reduced, whereas CD8⁺ cell count and CD11b⁺CD8⁺ cell count were increased postoperatively. Many kinds of alterations to cellular immunity have been previously identified in patients undergoing cardiac operations with CPB. Lymphocytopenia, caused mainly by a reduction of the helper/inducer (CD4⁺) lymphocyte occurred on POD 1 and lasted for several days after the operation, with values returning to the preoperative level on POD 7.^{1,2,4-8} There has been some controversy about the postoperative changes in the suppressor/cytotoxic (CD8⁺) lymphocyte count¹⁻⁹; however, most investigators have reported that the CD4⁺/CD8⁺ ratio was reduced postoperatively.^{2,3,5,6,8} In addition, NK cell activity^{3,4,6,21} and interleukin-2 production^{1,2,8} were found to be depressed after CPB. In vitro studies of the lymphocyte response to concanavalin A⁷ or phytohemagglutinin,^{2,4,7,8} as well as an in vivo study of delayed-type hypersensitivity to antigens,¹ also demonstrated impaired responses after CPB.

To our knowledge, this is the first study that has reported changes in the post-CPB functional subset of suppressor T lymphocytes and NK cells with use of a two-color analysis simultaneously with NK cell activity analysis. When functional subsets of lymphocytes are stained with a single monoclonal antibody, it is difficult to identify specific functional types of lymphocytes. With a two-color analysis, we

Fig. 2. Changes in (a) the percentage of NK cells (CD56⁺CD16⁺ cell) in peripheral blood and (b) their absolute count before and after CPB. There were no significant differences in intergroup comparison ($p > 0.05$).

Fig. 3. NK cell cytotoxic activity before operation and on various days after CPB. Asterisks indicate statistical differences in intergroup comparison ($p = 0.02$).

were able to more accurately ascertain the counts of suppressor T lymphocytes and NK cells than can be done with a single-color analysis.

Cell-mediated immunity is considered to be important for the prophylaxis of postoperative infections.^{1, 2, 3, 20} Postoperative complications such as septic shock or mediastinitis usually occur as a result of bacterial infection. To counteract microorganisms, phagocytic cells such as monocytes, macrophages, and polymorphonuclear neutrophils play an important role. Cytokines released by T cells activate these phagocytes to destroy the materials they

have internalized.²² Therefore impairment of cellular immunity after CPB may lead to infectious complications. In fact, from a clinical perspective, Markewitz and associates¹ have reported that impaired immunoreactivity increases the risk of septic multiorgan failure after cardiac operations involving CPB.

Meanwhile, cimetidine, a histamine type 2 receptor antagonist, has been known to enhance cell-mediated immune responses in vivo and in vitro.^{11-21, 23} Several reports have demonstrated the effectiveness of cimetidine in the treatment of various malignancies unresponsive to customary treatments, including remarkable regression of malignant melanoma²⁴ and improved survival in patients with gastric cancer^{18, 19} and colorectal cancer.^{16, 17} In addition, it has been reported that administration of cimetidine improved survival after sepsis in burned mice.²⁰ In consideration of these reports, we previously investigated the immunomodulating effects of cimetidine on post-CPB cellular immunity, especially in relation to NK cell activity, and we revealed that cimetidine preserved NK cell activity after cardiac operations.²¹

With the perioperative use of cimetidine in our latest study, the NK cell activity on POD 1 was again shown to have been significantly preserved. Organisms such as bacteria and viruses that succeed in entering tissues are rapidly exposed to phagocytic cells, principally polymorphonuclear leukocytes and macrophages. It has been realized in recent years

that macrophages perform many of their effects via secreted molecules, several of which show antimicrobial activity. Through these cytotoxic secretory molecules, macrophages can destroy Gram-positive bacteria. Meanwhile, in T cell-independent defense mechanisms, NK cells release interferon- γ , which augments the microbicidal potentials of macrophages and increases their adherence to endothelial cells.²⁵ Accordingly, we consider that the immunomodulatory effects of cimetidine on NK cell activity after CPB have clinical importance.

The correlation between net NK cell count ($CD56^+CD16^+$ cell) and NK cell activity after cardiac operations with CPB was revealed in this study with the use of a two-color analysis. Nguyen and colleagues^{3, 26} stated in their reports that post-CPB reduction of NK cell activity was a result of a decrease in the number of NK cells whose cytotoxic function remained intact. However, our data demonstrate that there are some discrepancies in the post-CPB change between NK cell activity and NK cell count (Fig. 2, *b*, and Fig. 3): NK cell activity was lowest on POD 1, whereas NK cell count was lowest on POD 3. In addition, from a morphologic perspective, De Angeli and colleagues⁷ clarified with scanning electronic microscopy that lymphocytes drastically lose their microvilli on the surface during CPB, and that microspheres connected to lymphocyte membranes were considerably reduced. Such a lymphocyte may lose its cytotoxic ability. These data suggest that NK cell cytotoxic activity may deteriorate after CPB. Because cimetidine did not affect the post-CPB NK cell count, we consider that cimetidine may have preserved the cytotoxic ability of the NK cells.

Another important finding of the present study is that post-CPB suppressor T cell activation was inhibited by cimetidine (Fig. 1). It has been previously reported that cimetidine inhibits the function of suppressor T lymphocytes.^{13, 14} This subset of lymphocytes regulates the immune responses of helper T lymphocytes, which play a central role in cell-mediated immunity. The helper T lymphocyte modulates various types of cellular cooperation and produces a wide variety of cytokines. Inhibition of suppressor T lymphocytes with cimetidine, therefore, may be beneficial to patients because it may preserve the cell-mediated immune response after CPB.

It is well known that suppressor T lymphocytes have a receptor for histamine, which has inhibitory effects on cellular immunity.²⁷ It has been reported

that these suppressive effects of histamine are mediated through the histamine type 2 receptor.²³ Therefore histamine type 2 receptor antagonists may have an immunomodulatory effect. Hahm and coworkers¹⁵ demonstrated that cimetidine had the strongest immunomodulative effect compared with that of other histamine type 2 receptor antagonists that have a much stronger antihistaminic potency. In consideration of these findings, it remains unclear whether the effect of cimetidine in preserving NK cell activity is a result of the impairment of suppressor T lymphocytes or other unknown mechanisms, which may be related to the structural features of cimetidine.

In summary, we have demonstrated that patients undergoing cardiac operations exhibited an alteration in the peripheral lymphocyte subsets: the percentages of $CD3^+$ cells and $CD4^+$ cells were decreased and those of $CD8^+$ cells were increased. There was no significant alteration in the $CD11b^+CD8^+$ cell count (net suppressor T cell count). We also demonstrated that NK cell cytotoxic activity and its net count ($CD56^+CD16^+$ cell count) were decreased after CPB. With the perioperative administration of cimetidine, post-CPB deterioration of NK cell activity was diminished and the suppressor T lymphocyte count was reduced. Although the number of patients included in this study was too small to permit evaluation of the clinical effectiveness of cimetidine, our study raises the possibility that perioperative administration of cimetidine preserves cell-mediated immunity and prevents infectious complications associated with cardiac operations.

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