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The preoperative administration of lentinan ameliorated the impairment of natural killer activity after cardiopulmonary bypass 體外循環

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Abstract

The aim of this study was to determine whether the preoperative administration of lentinan, which is used clinically to activate T cell function in cancer patients, prevents the impairment of lymphocyte function after cardiopulmonary bypass (CPB). A total of 25 adults undergoing coronary artery bypass 冠狀動脈繞道手術 grafting were enrolled in this study. Lentinan (2 mg) was given to 10 randomly selected patients 7 d before surgery, while the other 15 patients were considered as a control. The white blood cell count, percentage of lymphocytes, subsets of lymphocytes, and natural killer cell activity were measured preoperatively, immediately after CPB and 1, 3, and 6 d after surgery.

The white blood cell counts and the percentage of lymphocytes were not significantly different between the two groups; however, the percentage of CD4-positive cells in the lentinan group recovered to normal more rapidly than in the control group. Although natural killer cell activity was impaired in the control group after CPB, it maintained a nearly normal level in the lentinan group. The preoperative administration of lentinan for patients undergoing CPB ameliorated the impairment of natural killer activity and promoted the rapid recovery of CD4-positive cells. © 1999 International Society for Immunopharmacology. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Cardiac surgery; Cardiopulmonary bypass; Immunity
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1. Introduction

Open heart surgery is now performed relatively safely, especially since most operations are carried out under cardiopulmonary bypass (CPB). However, CPB has several demerits, such as complement activation [5] and fluid retention in the third space [28]. Moreover, hypothermia associated with CPB stimulates an adrenergic response and catecholamine release [17] and low blood flow disrupts the endocrine system leading to hyperglycemia and abnormal mineral homeostasis [31]. One of the most serious problems associated with CPB is impairment of the patient's immunity [34,35] and several papers have been published on the impairment of cellular [34] and humoral [35] immunity. Impaired immunity can lead to postoperative infection and postoperative graft-vs-host disease [25]. Postoperative infection following cardiac surgery is more critical than in general surgery because the operative stress is large and can lead to multiple organ failure easily when infection is associated and artificial prosthesis sometimes used. However, to our knowledge, only one study on preventing this impairment by combined indomethacin and thymopentin treatment has been reported [24].

In the present study, we focused on lentinan [7], a drug used clinically to improve the immunity of patients with cancer in Japan. Lentinan is a fully purified β-1,3-D-glucan with β-1,6 branches. It was first discovered by Chihara et al. [7], who isolated it from the hot-water extract of the fruit body of Lentinus edodes (Berk.) Sing., the most popular edible mushroom in Japan. The molecule stimulates both T-cell-dependent and -independent immune mechanisms [14,23] and causes the complete regression of sarcoma 180 cells transplanted subcutaneously in mice. Suppression of the helper T cell response in sarcoma 180 tumor-bearing mice is restored by lentinan [13]. Natural killer cell activity is enhanced after lentinan injection in patients with a variety of malignancies [26]. Clinically, a synergistic effect of lentinan with chemotherapeutic agents has been reported in patients with cancer [11,32]. Lentinan does not have a direct effect on tumor cells, but prevents the impaired host cellular immunity caused by cancer [15]. Lentinan exerts no direct cytocidal action on normal cells, unlike most chemotherapeutic agents that often produce detrimental effects [15]. Thus, as lentinan can safely be administered to the majority of patients, with very few side effects [11], we employed it in an attempt to prevent the impairment of lymphocyte function after CPB.

2. Materials and methods

2.1. Patients and study protocol

The subjects of this study were 25 patients undergoing coronary artery bypass grafting. Each patient gave informed consent and the Medical Ethics Committee of the Yamaguchi University School of Medicine approved this clinical study. All patients were clinically stable upon entry into the study, without any evidence of infection or organ failure. Ten patients who were randomly selected as the lentinan administration group were given 2 mg lentinan intravenously 7 d before their operation, which is a standard dose and timing of the treatment for cancer patients [11,33]. The other 15 patients were considered as a control group and given saline as a placebo intravenously using the same regimen. The characteristics and operative details of both

Table 1
Background of the patients (categoric data are presented as the actual numbers, data are presented as mean ± standard deviation; LVEF = left ventricular ejection fraction; Redo = redo operation; NS = not significant)

	Lentinan group	Control group	*P*
N	10	15	
Age (years)	67 ± 10	62 ± 10	NS
Sex (M/F)	8:2	10:5	NS
Diabetes mellitus	3	4	NS
Hypertension	6	7	NS
Hyperlipidemia	1	1	NS
Smoking history	4	6	NS
Previous infarction	6	6	NS
LVEF < 0.40	2	1	NS
Redo	2	1	NS

groups are outlined in Tables 1 and 2. For the CPB circuit, a centrifugal pump (Bio-Console; Bio-Medicus, Inc, Eden Prsrie, MN) and a fiber oxygenator (MERA EXCELUNG αHPO-25RHF, SENKO MEDICAL INSTRUMENT Co., Ltd, Tokyo, Japan) were used. The pump, circuit, and oxygenator were primed with 3 ml kg^{-1} 20% mannitol, 3 mg kg^{-1} betamethasone sodium phosphate (Rinnderon), Ringer's lactate solution, and heparinized blood, 100 units per 100 ml, if required. The total priming volume was 1.8 l, with an ideal dilution rate of 20–25% and a perfusion index of 2.4 l min^{-1} m^{-2} body surface area. An initial prebypass bolus dose of heparin, 3 mg kg^{-1}, was infused to maintain an activated clotting time of more than 400 s during bypass. The operative procedure was performed under whole-body mild hypothermia with a rectal temperature maintained at between 31 and 33°C. A tepid blood cardioplegic solution was intermittently administered through the aortic root. After CPB, a dose of protamine sulphate equal to the heparin dose was administered.

Table 2
Operative details (data are presented as mean ± standard deviation; LITA = left internal thoracic artery; NS = not significant)

	Lentinan group	Control group	*P*
N	10	15	
Operation time (min)	477 ± 93	448 ± 78	NS
Total no. of distal anastomosis	2.30 ± 0.48	2.33 ± 0.49	NS
Usage of LITA	100%	100%	NS
Pump time (min)	163 ± 49	170 ± 47	NS
Clamp time (min)	103 ± 42	101 ± 28	NS
No. of transfused patients	4	6	NS
Blood transfusion (ml)	518 ± 801	607 ± 506	NS

2.2. Sample collection

Blood samples for measuring WBC count, the percentage of lymphocytes, and the lymphocyte subsets and function were obtained via an arterial line that had been placed in the radial artery. If the arterial line was removed, blood samples were obtained from a femoral artery. Blood samples were collected preoperatively, immediately after CPB, and 1, 3, and 6 d postoperatively.

WBC counts and the percentage of lymphocytes in the blood were measured using an automated cell counter (Sysmex K-1000, Toa Medical Electronics, Co, Ltd, Kobe, Japan).

2.3. Measurement of the lymphocyte subsets

CD4- and CD8-positive cells were measured by flow cytometric analysis using OKT4 (Ortho Diagnostics Inc, USA) and OKT8 (Ortho Diagnostics Inc, USA) monoclonal antibodies, respectively.

2.4. Measurement of the natural killer cell activity

Natural killer cell activity was measured by a ^{51}Cr releasing assay using ^{51}Cr-labelled K-562 cells (16). Natural killer cell activity was calculated from the radioactivity of ^{51}Cr in the supernatant and is expressed as: percentage lysis = (experimental release − spontaneous release)/(maximum release − spontaneous release) × 100%, with maximum release being defined as the total radioactivity in the target cells.

2.5. Statistics

Categoric data are presented as means ± the standard deviation. Statistical analysis of categoric variables was performed on cross-tables using the Pearson's χ^2 test. The WBC counts, the percentage of lymphocytes, the percentage of CD4- and CD8-positive T cells, the stimulatory index, and the natural killer activity are presented as means ± the standard error of the mean. The kinetic data in the two groups were first analyzed by the repeated measure analysis of variance (ANOVA). Student's *t* test was performed to compare values between the two groups *(Stat View 4.5)*. A *P* value of <0.05 was considered to be significant.

3. Results

3.1. Background of the patients

The mean age and the male to female ratio in both groups were not significantly different. The associated percentage of patients with diabetes mellitus, hypertension, hyperlipidemia, smoking history, previous myocardial infarction, poor left ventricular function (ejection fraction less than 40%), and redo operation in both groups were also not significantly different (Table 1).

3.2. Operative details

The mean operation time was 477 ± 93 min in the lentinan group and 448 ± 78 min in the control group. The mean perfusion time was 163 ± 49 min in the lentinan group and 170 ± 47 min in the control group. The mean aorta clamp time was 103 ± 42 min in the lentinan group and 101 ± 28 min in the control group. Four patients were transfused homologous blood in the lentinan group, six patients in the control group. The mean volume of homologous blood transfused was 518 ± 801 ml in the lentinan group and 607 ± 506 ml in the control group. There were no significant differences between the two groups in any of these parameters (Table 2).

All patients not accompanied with serious complications such as infection or graft-vs-host-disease were discharged from hospital.

3.3. Changes in white blood cell counts and the percentage of lymphocytes

The WBC counts increased postoperatively, peaking 3 d after the operation in both groups at 15,400 ± 1181 mm^{-3} in the lentinan group and 13,543 ± 1101 mm^{-3} in the control group, with no significant difference during the period of measurement between the two groups (Fig. 1(a)). The percentage of lymphocytes decreased postoperatively in both groups, being lowest 1 d after the operation in both groups at 6.3 ± 1.3% in the lentinan group and 9.4 ± 1.7% in the control group; thereafter, gradually recovering. There was no significant difference during the period of measurement between the two groups (Fig. 1(b)).

3.4. Changes in CD4- and CD8-positive T cells

The number of CD4-positive T cells decreased postoperatively in both groups with the lowest count 1 d after the operation, at 26.0 ± 3.5% in the lentinan group and 22.8 ± 2.0% in the control group. The CD4-positive T cells in the lentinan group recovered to a normal value of more than 35% 3 d after the operation, but those in the control group did not recover. The number of CD4-positive T cells in the lentinan group was significantly ($P = 0.004$) higher than that in the control group 3 d after the operation (Fig. 2(a)). The number of CD8-positive T cells was also decreased postoperatively in both groups with the lowest count 3 d after the operation, at 18.4 ± 1.7% in the lentinan group and 13.8 ± 1.6% in the control group. There was no significant difference between the two groups at any time point (Fig. 2(b)).

3.5. Changes in natural killer cell activity

The natural killer cell activity, the normal range of which is from 18 to 50%, was also decreased postoperatively in both groups. The natural killer cell activity in the lentinan group reached its lowest level 1 d after the operation, but was only marginally reduced to 17.1 ± 2.9% while that in the control group reached its lowest level of 8.4 ± 0.9% 3 d after the operation. The natural killer cell activity in the lentinan group was significantly higher than that in the control group 3 ($P = 0.0039$) and 6 d ($P = 0.035$) after the operation (Fig. 3).

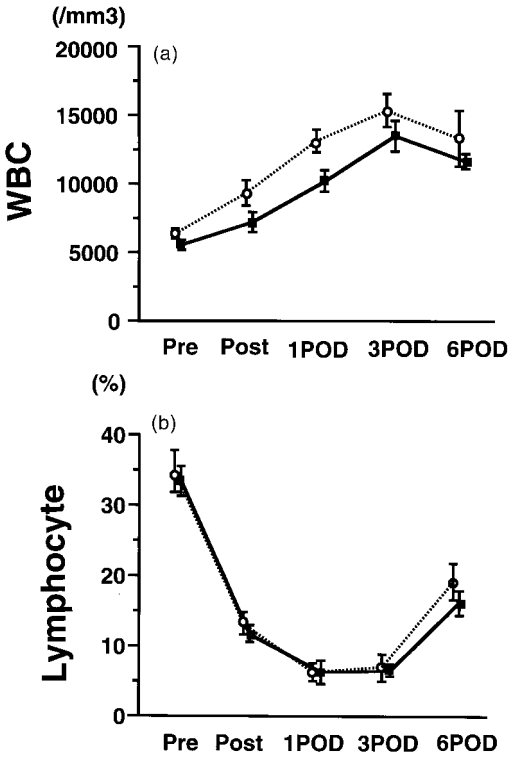

Fig. 1. Changes in white blood cell counts (a) and the percentage of lymphocytes (b). The WBC counts increased postoperatively in both groups and the percentage of lymphocytes decreased postoperatively in both groups. There was no significant difference in WBC counts and the percentage of lymphocytes during the period of measurement between the two groups: lentinan group,–– –○––; control group,—— ■—— .

4. Discussion

The results of this study demonstrate that CPB causes alterations in cell-mediated immune response. Previous studies have shown that the capacity of the immune system is reduced in patients who have undergone cardiac operations due to a waste of complement factors [5,16], and a decrease in cellular elements of the innate immune system, such as, white blood cells or natural killer cells [29,30]. Nevertheless, to the best of our knowledge, there has been only one attempt made to restore the impaired immune response caused by CPB [24]. In that study, combined treatment with indomethacin [8–10] and thymopentin [12,36] was used in the hope that it would counteract the dysregulation of cell-mediated immune response to block PGE_2. Indomethacin, a non-steroidal anti-inflammatory drug, blocks the synthesis of PGE_2 via cyclooxygenase inhibition [8–10] and thymopentin, the synthetic thymus hormone, enhances T cell proliferation and maturation [12,36]. According to the results of the previous study [24], indomethacin and thymopentin restored the $CD4^+$ and $IL\text{-}2R^+$ cell counts after CPB, and the serum levels of IL-1, IL-2, and γ-interferon were higher than those of the no treatment group. Since this report, no further studies on this treatment have been published.

We employed lentinan in an attempt to prevent the impairment of immunity after CPB.

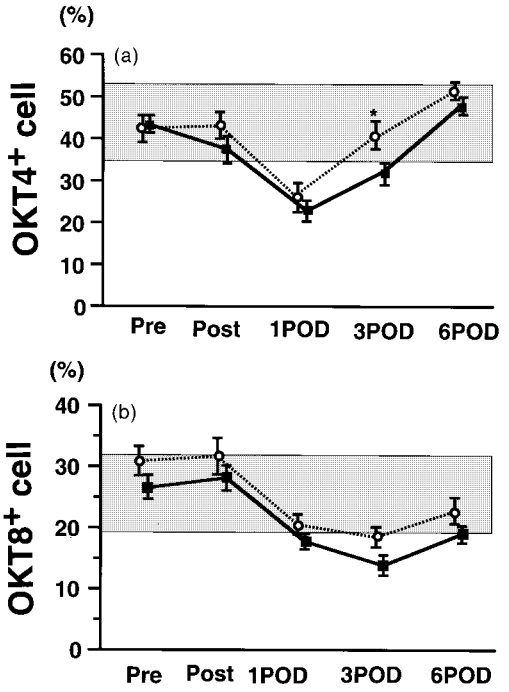

Fig. 2. Changes in CD4-(a) and CD8-(b) positive T cells. The CD4-positive T cells decreased postoperatively in both groups. The CD4-positive T cells in the lentinan group recovered to a normal value 3 d after the operation, but that in the control group did not recover. The number of CD4-positive T cells in the lentinan group was significantly higher than that in the control group 3 d after the operation. The number of CD8-positive T cells was also decreased postoperatively in both groups. There was no significant difference between the two groups during the period of measurement. lentinan group,— –○—; control group,—— ■——; normal range, ■; $^{*}P < 0.05$.

Lentinan is currently used in Japan to activate the immunity of cancer patients and positive clinical data has been obtained in this field [27]. Although the mechanisms of lentinan have not been fully investigated, it is known that it sends signals to T cells and macrophages to be

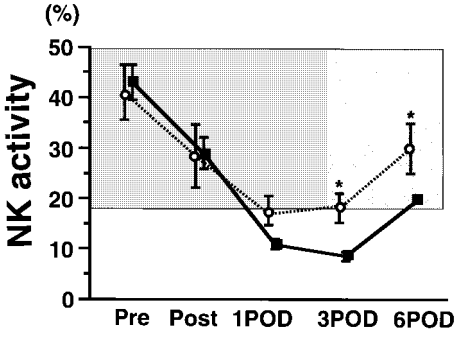

Fig. 3. Changes in natural killer cell activity. The natural killer activity was decreased postoperatively in both groups. The natural killer activity in the lentinan group was significantly higher than that in the control group 3 and 6 d after the operation: lentinan group,— –○—; control group,—— ■——; normal range, ■; $^{*}P < 0.05$.

activated within 4 d after its administration [20–22]. The activated T cells and macrophages accelerate the activation of the host immune system which then attacks cancer. As lentinan has no direct killing action on cancer cells or normal cells, side effects are minimal. It was reported from a previous clinical trial for cancer patients that the percentage of side effects was 5.6% and those were not serious and transient [11]. In our clinical protocol, 2 mg of lentinan was administered 7 d before surgery. Previous study showed that lymphokine-activated killer (LAK) activity and NK activity was significantly enhanced 7 d after a single injection [3]. Further, the same clinical protocol for gastric cancer patients showed the good immune enhancing effect previously [11,33]. However, 1 mg of lentinan administered twice a week or repeated administration also showed increased NK activity [1,2]. Therefore, repeated administration might be an alternative method.

Our data showed that the percentage of lymphocytes was reduced after CPB with no significant difference between the two groups; however, NK activity in the lentinan group was maintained nearly within the normal range whereas that in the control group was significantly impaired. Moreover, $CD4^+$ T cells, which are the essential cellular element of forward regulation, recovered much more rapidly in the lentinan group than in the control group. The administration of lentinan did not completely prevent the impairment of patients' immunity; however, it is extremely important to ensure that normal NK activity is maintained.

Although cardiac surgery is becoming safe, the numerous disadvantages of CPB should be borne in mind [5,17,28]. Minimally invasive direct coronary bypass [4] can be performed without the use of CPB, but CPB is necessary for the majority of cardiac operations. In situations when CPB must be used, we have to try to reduce the associated risks. Impairment of patients' immunity is one of the prominent disadvantages that can easily lead to infection and graft-vs-host disease. For example, wound infection after cardiac surgery developed to mediastinitis and sternal dehiscence at an incidence of 1–2% [18,19]. The mortality rate after this complication was reported to be from 6–70% [6] and is considered serious.

In conclusion, the preoperative administration of lentinan for patients undergoing CPB ameliorated the impairment of natural killer activity and promoted the rapid recovery of $CD4^+$ cells.

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