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# Preventive Effect of a Traditional Herbal Medicine, Hochu-ekki-to, on Immunosuppression Induced by Surgical Stress

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**Abstract**

***Purpose.*** To examine the effect of preoperative administering of a Japanese traditional herbal medicine, Hochu-ekki-to (TJ-41), on immunosuppression induced by surgical stress in patients with gastrointestinal malignancies.

***Methods.*** To monitor the immune functions, the mitochondrial membrane potential (MMP) and natural killer (NK) cell activity prior to and following operation were measured in peripheral blood lymphocytes (PBL) in patients with ($n$ = 20) or without ($n$ = 27) the preoperative administering of TJ-41 for 7 days. The plasma catecholamine and interleukin (IL)-6 levels were also analyzed prior to and following the operation.

***Results.*** The numbers of MMP-high CD56-positive cells (NK cells) and NK cell activities in the TJ-41-treated group were significantly higher than those in the control group ($P$ = 0.026 and $P$ = 0.037, respectively). An elevation of plasma noradrenaline and IL-6 following surgery was also inhibited by the preoperative administering of TJ-41 ($P$ = 0.023 and $P$ = 0.039, respectively). A positive correlation between MMP-high CD56-positive cell numbers and NK cell activity in PBL treated with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) in vitro suggested that MMP measurement in CD56-positive cells can serve as a convenient alternative to evaluate the NK cell activity.

***Conclusion.*** Our findings suggest that the preoperative administering of TJ-41 prevents surgical stress-induced immunosuppression by maintaining the NK cell activity and inhibiting the elevation of stress mediators.

**Key words** Herbal medicine · Natural killer cell · Surgical stress · Mitochondrial membrane potential

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## Introduction

It has been suggested that the immunosuppression induced by surgical stresses promotes either recurrence or distant metastasis in cancer patients after surgery.[1,2] An immune dysfunction induced by surgical stresses has been also reported to be associated with postoperative complications, such as surgical site infections.[1] For example, the postoperative impairment of natural killer (NK) cells affects early recurrence and metastasis of cancer and postoperative complications.[3,4] In the previous studies, we have demonstrated that a reduction in mitochondrial membrane potential (MMP) can be detected in the peripheral blood lymphocytes (PBL) undergoing apoptotic processes, and that the monitoring of MMP in PBL, mainly in CD56-positive NK cells, following surgery may be one of the useful markers for estimating the magnitude of surgical stresses on the immune system.[5]

Hochu-ekki-to (TJ-41), a Japanese traditional herbal medicine that originated in China, has been clinically used to accelerate the recovery from severe illness and surgery since ancient times[6] and to prevent opportunistic infections in infection-prone patients in Japan.[7,8] Several reports have also been published describing the biological actions of TJ-41 on immune responses in mice. TJ-41 can induce the activation of peritoneal macrophages[9] and enhance the protection against bacterial, viral, or fungal infections.[10–13] In addition, TJ-41 has also been shown to augment the anti-tumor immune responses in mice both in vivo and in vitro, through the enhancement of NK cell activity and/or the production of cytotoxic cytokines, such as IFN-γ.[14–16]

Although accumulating evidence has shown the importance of TJ-41 in various immune functions in animal models, the roles of TJ-41 in humans have not been exactly defined. In the present study, we measured MMP of PBL before and after surgery in patients with gastrointestinal cancer with or without preoperative

administrating of TJ-41, to examine whether TJ-41 can modulate immunosuppression induced by surgical stresses. We demonstrated that the preoperative administering of TJ-41 was useful for maintaining NK cell activity following surgery. In addition, we showed that an elevation of stress mediators, such as plasma noradrenaline and interleukin (IL)-6, following surgery was inhibited by the preoperative administering of TJ-41. To our knowledge, this is the first study to show the crucial role of TJ-41 in preventing the immunosuppression induced by surgical stress.

## Methods

### *Hochu-ekki-to (TJ-41)*

TJ-41 is manufactured as a spray-dried powder of hot water extract obtained from 10 medicinal plants in the following ratio: Astragali Radix (4.0), Atractyloclis Lanceae Rhizoma (4.0), Ginseng Radix (4.0), Angelicae Radix (3.0), Bupleuri Radix (2.0), Zizyphi Fructus (2.0), Auranti Nobilis Pericarpium (2.0), Glycyrrhizae Radix (1.5), Cimicifugae Rhizoma (1.0), and Gingiberis Rhizoma (0.5) (Tsumura, Tokyo, Japan). The quality of this drug is controlled by measuring the contents by high-performance liquid chromatography (HPLC).[12,17]

### *Patients*

Between 2001 and 2005, 47 patients who underwent surgery at Kitano Hospital were divided into two groups: the TJ-41-treated group and the control group. The TJ-41-treated group comprised 20 patients who were administered 7.5 g (2.5 g × 3) TJ-41 every day for 7 days before operation. As for the control group with similar backgrounds, 27 patients without TJ-41 being administered were selected. There were no significant differences between the two groups concerning the characteristics of patients, including sex, age, preoperative body weight, surgical procedure, blood loss, duration of surgery, and blood transfusion (Table 1). The surgical procedures included intrathoracic esophagectomy for esophageal cancer, hepatic resection for liver metastasis of colon cancer or hepatocellular carcinoma (HCC), partial or total gastrectomy for gastric cancer, colectomy or anterior resection of the rectum for colorectal cancer, and pylorus-preserving pancreatoduodenectomy (PpPD) for pancreatic cancer. None of subjects had any viral or bacterial infection or received immunosuppressive agents such as corticosteroids, antibiotics, or anti-cancer agents during the 1-month period preceding the study. All the patients were given epidural analgesia by means of continuous administering of 0.15% bupivacaine hydrochloride and morphine hydrochloride (6 mg/100 ml of saline). Epidural analgesia was continued until postoperative day (POD) 2. General anesthesia was induced by intravenous administering of droperidol (0.5 mg/kg), fentanyl (4 g/kg), and thiopental (5 mg/kg). Tracheal intubation was facilitated with vecuronium (0.1 mg/kg), and anesthesia was maintained with 60%–65% nitrous oxide, 35%–40% oxygen, fentanyl (2 g/kg per hour), and vecuronium (40 g/kg per hour). Informed consent was obtained from all of the patients in accordance with the Helsinki Declaration,

**Table 1.** Characteristics of patients

| | Control | TJ-41 | *P* |
|---|---|---|---|
| Male : female | 18 : 9 | 13 : 7 | 0.848* |
| Age | 64 (44–79) | 64 (44–83) | 0.488** |
| Body weight (kg) | 55.8 (35.0–67.9) | 55.7 (37.4–82.0) | 0.490** |
| *Procedure* | | | 0.350*** |
| Esophagectomy | 5 | 3 | |
| Gastrectomy | | | |
| Total | 2 | 2 | |
| Partial | 5 | 4 | |
| Colectomy | | | |
| Colon | 5 | 1 | |
| Rectum | 2 | 3 | |
| Hepatectomy | | | |
| Lobectomy | 3 | 2 | |
| Partial | 3 | 4 | |
| PpPD | 2 | 1 | |
| Blood loss (ml) | 777 (20–2523) | 859 (31–1974) | 0.325** |
| Duration | 4:44 (1:10–10:35) | 4:45 (2:05–10:55) | 0.491** |
| Blood transfusion | 14 | 11 | 0.935* |

*Yates' Chi square test, **Student's *t* test, ***Analysis of variance

and the study was approved by the Ethics Committee of our hospital.

*Blood Samples*

Heparinized blood samples were obtained at 7:00 a.m. on the day prior to the surgical procedure (prior to and following the administering of TJ-41) and on POD 1. Lymphocytes were prepared with Ficoll Hypaque (Pharmacia, Uppsala, Sweden) and used for the experiments.

*Flow Cytometric Analyses of MMP*

Flow cytometry was performed with a FACS Calibur (Becton Dickinson, San Jose, CA, USA), as described earlier.[5,18] To measure MMP, cells were incubated for 20 min at 37°C with 1 nM 3,3-dihexyloxacarbocyanine iodide [$DiOC_6(3)$; Molecular Probes, Eugene, OR, USA], a cationic lipophilic fluorochrome that is widely employed for the cytofluorometric determination of MMP.[19] Some of the cells were then incubated for 30 min at 37°C with 40 μM carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP, Sigma, St. Louis, MO, USA), an uncoupling agent that eliminates MMP. The cells that showed a higher fluorescence than that in 95% of cells treated with CCCP were defined as MMP high. For the simultaneous assessment of MMP and surface markers, cells were first stained with $DiOC_6(3)$, followed by staining with phycoerythrin (PE)-labeled anti-human CD3 or anti-human CD56 antibodies (Becton Dickinson) for 30 min on ice. CD3-positive MMP-high and CD56-positive MMP-high cells were determined after 10 000 cells per sample were counted.

*NK Cell Activity*

The NK cell activity was measured using K562 cells in a 4-h standard $^{51}Cr$ release assay as described earlier.[20] The effector-to-target ratios used for this study were 40:1, 20:1, 10:1, and 5:1. The lytic units ($LU_{20}$) were calculated as the number of effector cells required to lyse 20% of the 10 000 target cells, and the results were presented as the number of units per $10^6$ effector cells.[21]

*Plasma Levels of Catecholamines and IL-6*

The plasma concentrations of catecholamines (adrenaline and noradrenaline) and IL-6 were measured in 19 patients in the TJ-41-treated group and 16 patients in the control group prior to surgery (prior to administering of TJ-41) and on POD 1. There were no significant differences between these two groups regarding the characteristics of patients (data not shown). Adrenaline (normal range below 100 pg/ml) and noradrenaline (normal range 100–450 pg/ml) were assessed by an HPLC method using electrochemical detection. The IL-6 concentration (normal range below 4.0 pg/ml) was determined by ELISA.[5]

*Statistical Analysis*

The results are presented as the mean and standard deviation. The qualitative data were analyzed by Yates' Chi square test or analysis of variance, and Student's *t* test was used to compare the quantitative data. A value of $P < 0.05$ was considered to be significant.

## Results

*Effect of TJ-41 on MMP of PBL and CD3-Positive Cells*

To estimate the magnitude of surgical stresses on the immune system, MMP was assessed in PBL prior to and following surgery with or without the preoperative administering of TJ-41. Postoperative numbers of MMP-high PBL were compared with preoperative numbers prior to the administering of TJ-41, which were regarded as 100%. Following surgery, the TJ-41-treated group and the control group showed a reduction of the MMP-high PBL numbers to approximately 75% and 70% of the preoperative values, respectively, and there was no significant difference between the two groups (Fig. 1A, $P = 0.236$).

To elucidate the effects of TJ-41 on T cells, the percentages of MMP-high CD3-positive cells were evaluated. The postoperative reduction in MMP-high CD3-positive cell numbers was approximately 30% following surgery in both groups, and there was no significant difference between the two groups (Fig. 1B, $P = 0.368$).

*Effect of TJ-41 on MMP of CD56-Positive Cells*

To clarify the effects of TJ-41 on NK cells, the percentages of MMP-high CD56-positive cells were evaluated. The numbers of MMP-high CD56-positive cells in the TJ-41-treated group decreased to approximately 77% of the preoperative numbers, whereas those in the control group decreased to approximately 55%. There was a significant difference between the two groups (Fig. 2A, $P = 0.026$).

*Effect of TJ-41 on NK Cell Activity*

The NK cell activity, as measured by a conventional $^{51}Cr$ release assay using K562 cells as a target, was analyzed

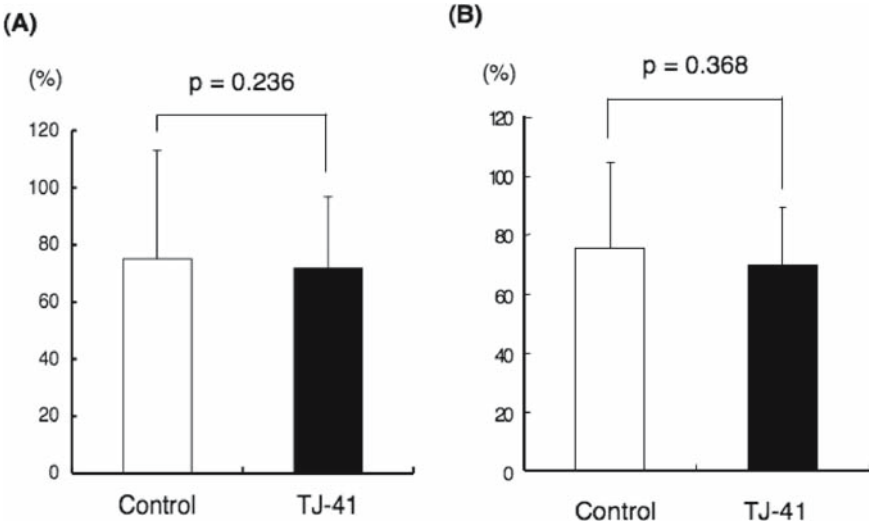


**Fig. 1.** Effect of TJ-41 on the number of mitochondrial membrane potential (MMP)-high peripheral blood lymphocytes (PBL) and MMP-high CD3-positive cells. The postoperative numbers of MMP-high PBL (**A**) and MMP-high CD3-positive T cells (**B**), which were calculated as the percentage relative to the preoperative numbers before administering TJ-41, are shown in TJ-41-treated and control groups

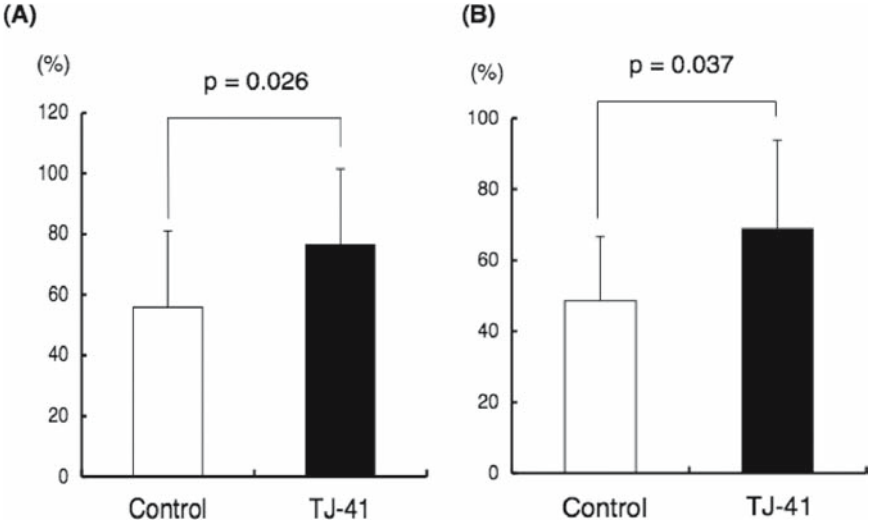


**Fig. 2.** Effect of TJ-41 on the number of MMP-high CD56-positive cells and natural killer (NK) cell activity. **A** The postoperative numbers of MMP-high CD56-positive cells (NK cells), which were calculated as the percentage relative to the preoperative numbers before administering TJ-41, are shown in TJ-41-treated and control groups. **B** Postoperative NK cell activities, which were measured by conventional $^{51}Cr$ release assay using K562 cells as a target and calculated as the percentage relative to the preoperative activities before administering TJ-41, are shown in the TJ-41-treated and control groups

on the days prior to and following the surgical procedure, to determine whether a reduction in the MMP levels in CD56-positive cells affects their cellular function. The NK cell activity in the TJ-41-treated group decreased to approximately 68% of the preoperative value, whereas that in the control group decreased to approximately 47%. Consistent with the results in the MMP-high CD56-positive cell numbers, a significant difference was observed between the two groups (Fig. 2B, $P = 0.037$).

We measured the MMP and NK cell activity after treating PBL with the uncoupling agent CCCP, to show the direct relationship between the reduction of MMP-high CD56-positive cells and NK cell activity in vitro. As shown in Fig. 3, there was a positive correlation between MMP-high CD56-positive cell numbers and NK cell activity, thus indicating that MMP-high CD56-positive cell numbers are able to reflect NK cell activity.

### *Effect of TJ-41 on MMP in CD56-Positive Cells and NK Cell Activity Before Surgery*

Mitochondrial membrane potential in CD56-positive cells and NK cell activity were measured in the blood samples obtained from seven patients in the TJ-41-treated group prior to and following the administering of TJ-41 (prior to surgery) to elucidate whether TJ-41 affects MMP and NK cell activity under unstressed conditions without a surgical procedure. As shown in Fig. 4, the administering of TJ-41 increased the number of MMP-high CD56-positive cells and NK cell activity

slightly, but not significantly ($P = 0.274$ and $P = 0.267$, respectively). This finding suggests that the administering of TJ-41 can prevent the reduction in MMP-high CD56-positive cells and NK cell activity under stresses induced by surgery, but does not affect MMP-high CD56-positive cell numbers and NK cell activity under unstressed conditions without surgery.

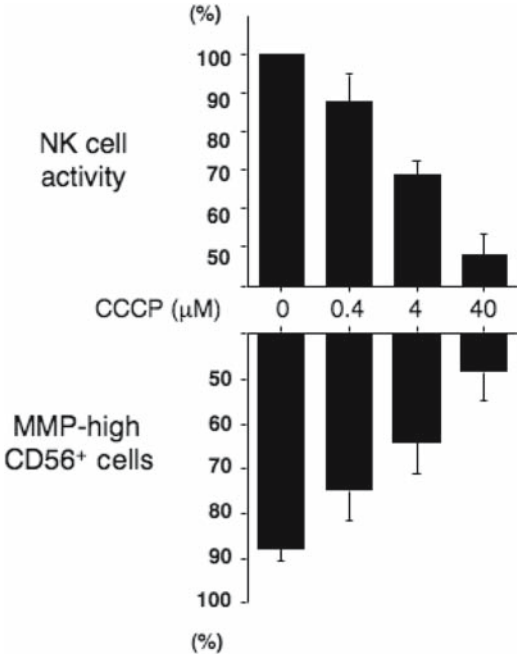


**Fig. 3.** Mitochondrial membrane potential-high CD56-positive cell numbers correlate well with NK cell activity. Percentages of MMP-high CD56-positive cells and NK cell activities were measured after treating PBL with the uncoupling agent carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) for 30 min at 37°C in vitro. The NK cell activity without CCCP treatment was regarded as 100%

*Effect of TJ-41 on Plasma Catecholamines and IL-6*

The plasma levels of stress-induced humoral factors, catecholamines (adrenaline and noradrenaline) and IL-6, were also measured before and after surgery to investigate the effects of the preoperative administering of TJ-41 on these stress mediators. The increase in the plasma adrenaline level was not different between TJ-41-treated and control groups (data not shown). However, the increase in the noradrenaline and IL-6 levels in plasma was significantly inhibited by the preoperative administering of TJ-41 (Fig. 5, $P = 0.023$ and $P = 0.039$, respectively).

## Discussion

There is accumulating evidence that surgical stress causes immune dysfunction, which may be related to an enhanced risk of infections as well as possible spread of residual cancer cells (micrometastasis) in cancer patients during and/or after operation.[1,2] Especially, a postoperative dysfunction of NK cells has been suggested to increase postoperative complications, such as surgical site infections, and to accelerate both the early recurrence and metastasis of cancer,[3,4] because NK cells are known to be central in the innate immune responses against infectious agents and tumors.[22] Indeed, we and others have demonstrated that the number of NK cells and/or NK cell activity is reduced by surgical stresses following operation.[5,23–25] The present study shows that the preoperative administering of TJ-41 can inhibit the reduction in NK cell activity following surgery. Although this finding suggests the possibility that the preoperative administering of TJ-41 may prevent postoperative infections and tumor recurrence, we could not show the preventive effects of TJ-41 on postoperative complications and tumor recurrence in the present study (data not

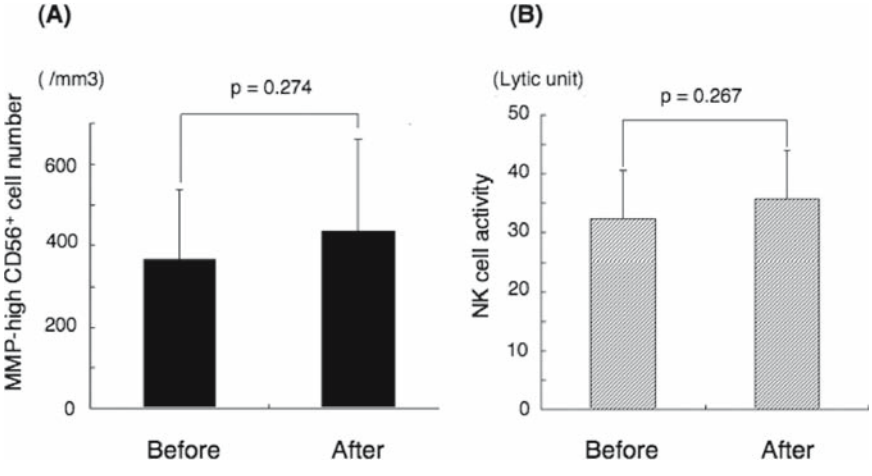


**Fig. 4.** The administering of TJ-41 did not significantly increase MMP-high CD56-positive cell number and NK cell activity before operation. MMP in CD56-positive cells (**A**) and the NK cell activity (**B**) were measured in the blood samples obtained from seven patients in the TJ-41-treated group before and after the administering of TJ-41 (before operation)

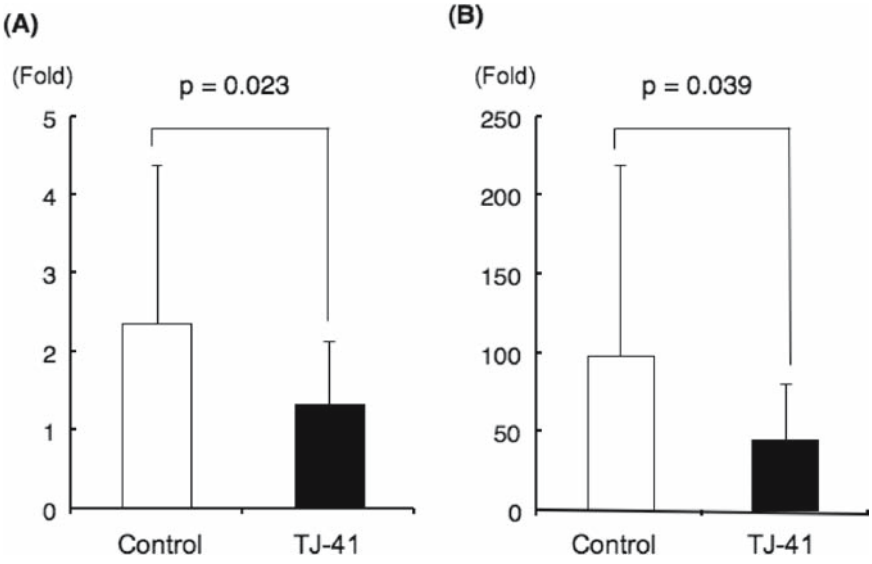


**Fig. 5.** Effect of TJ-41 on plasma noradrenaline and interleukin (IL)-6 concentrations. The postoperative values of noradrenaline (**A**) and IL-6 (**B**), which were calculated as the fold increase relative to the preoperative values before administering TJ-41, are shown in the TJ-41-treated and control groups

shown), possibly due to the limitations in the patient number and the observation period. Further study will thus be needed to clarify the therapeutic benefit of TJ-41.

TJ-41 is one of the most popular traditional herbal medicines in Japan, and is manufactured as a spray-dried preparation of hot water extract of a mixture consisting of 10 different kinds of medicinal plants specified in the Japanese Pharmacopeia at certain proportions. TJ-41 contains various substances, and clinical effects of TJ-41 are considered to be based on the combined effects of these components. TJ-41 has long been used as a drug that enhances digestive function and maintains homeostasis in the body.[6] Recently, several reports have been published describing the biological actions of TJ-41 on immune responses in animal models. For example, TJ-41 enhances NK cell activity,[14–16] activates anti-cancer immunity,[26] and exerts defense against infections[10–13] in mice. However, in humans, only a limited number of findings have so far been reported; TJ-41 eliminates Methicillin-resistant Staphylococcus aureus (MRSA) by improving nutritional status of patients with asymptomatic MRSA bacteriuria,[7] and maintains production of IFN-γ in patients with mycosis fungoides.[8] This study showed that TJ-41 can maintain the number of MMP-high NK cells following surgery, thus suggesting that TJ-41 may prolong the survival of NK cells through the prevention of apoptotic cell death under stressed conditions. Although we could not clarify the effects of TJ-41 on NK cells under unstressed conditions prior to surgery, Kuroiwa et al.[27] reported an inconsistent result that elderly patients complaining of general fatigue or weakness show enhanced NK cell activity following the administering of TJ-41 for at least 120 days. The discrepancy between this result and ours is probably due to differences in the treatment periods and/or patient population.

Surgical procedures induce systemic biological responses through the stimulation of the hypothalamic–pituitary–adrenal axis, as well as the autonomic nervous systems.[28] The communication circuit between the immune system and the neuroendocrine system has been reported to be mediated through ligands and receptors, which are shared in both systems.[29] Various types of surgical stress are suggested to activate the autonomic nervous system and the hypothalamic–pituitary–adrenal axis, thus leading to a dysfunction of the immune system by interfering with immune cell distribution, migration, and activation. We have demonstrated earlier that the reduction of MMP in NK cells induced by surgical stress may be mediated by the increase in plasma noradrenaline following major surgery.[5] Because the increase in plasma noradrenaline and IL-6 following surgery was also inhibited by the administering of TJ-41 in the present study, the effects of TJ-41 on NK cells may thus be explained, at least in part, by its regulation of the neuroendocrine system, which modulates release of humoral mediators, such as catecholamines and inflammatory cytokines. To elucidate the precise role of TJ-41 on the immune system, a complex neuroendocrine and cytokine network linking NK cell biology (growth, differentiation, and survival) should be investigated in the future.

The most commonly used assay for the analyses of NK cell cytotoxicity is based on the measurement of radioactive $^{51}Cr$ released by target cells.[20,21] However, it has a number of critical drawbacks including the use of radioactive compounds, high cost, and the need for

special laboratory equipment. The present study has shown a close relationship between NK cell activity and the percentages of MMP-high CD56-positive cells (Fig. 3). This finding suggests that the measurement of MMP in CD56-positive cells can serve as a convenient and rapid alternative to evaluate NK cell activity.

We have demonstrated that the preoperative administering of TJ-41 is capable of maintaining NK cell activity and inhibiting the elevation of stress mediators following major surgery. However, the present study has several limitations. For example, we could not show the clinical benefits of TJ-41, possibly because of a nonrandomized pilot study with small patient groups, which consisted of heterogeneous populations with different diseases and different surgical procedures. Therefore, as a further step, it will be important to plan larger scale randomized control studies with homogeneous patient populations to clarify the clinical benefits of TJ-41 pretreatment in patients with surgical stress.

## References


1. Hirai T, Matsumoto H, Yamashita K, Urakami A, Ikai K, Yamamura M, et al. Surgical oncotaxis — excessive surgical stress and postoperative complications contribute to enhancing tumor metastasis, resulting in a poor prognosis for cancer patients. Ann Thorac Cardiovasc Surg 2005;11:4–6.
2. Shakhar G, Ben-Eliyahu S. Potential prophylactic measures against postoperative immunosuppression: could they reduce recurrence rates in oncological patients? Ann Surg Oncol 2003; 10:972–92.
3. Ben-Eliyahu S, Page GG, Yirmiya R, Shakhar G. Evidence that stress and surgical interventions promote tumor development by suppressing natural killer cell activity. Int J Cancer 1999;80: 880–8.
4. Shakhar G, Ben-Eliyahu S. In vivo beta-adrenergic stimulation suppresses natural killer activity and compromises resistance to tumor metastasis in rats. J Immunol 1998;160:3251–8.
5. Takabayashi A, Kanai M, Kawai Y, Iwata S, Sasada T, Obama K, et al. Change in mitochondrial membrane potential in peripheral blood lymphocytes, especially in natural killer cells, is a possible marker for surgical stress on the immune system. World J Surg 2003;27:659–65.
6. Kuroiwa A, Liou S. Hochu-ekki-to. J Integr Med 2005;15:79–81.
7. Nishida S. Effect of Hochu-ekki-to on asymptomatic MRSA bacteriuria. J Infect Chemother 2003;9:58–61.
8. Tokura Y, Sakurai M, Yagi H, Furukawa F, Takigawa M. Systemic administration of Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), a Japanese–Chinese herbal medicine, maintains interferon-gamma production by peripheral blood mononuclear cells in patients with mycosis fungoides. J Dermatol 1998;25:131–3.
9. Kumazawa Y, Takimoto H, Miura S, Nishimura C, Yamada A, Kawakita T, et al. Activation of murine peritoneal macrophage by intraperitoneal administration of traditional Chinese herbal medicine, Xiao-Chai-Hu-Tang (Japanese name: Shosaiko-To). Int J Immunopharmacol 1998;10:395–403.
10. Li XY, Takimoto H, Miura S, Yoshikai Y, Matsuzaki G, Nomoto K. Effects of a traditional Chinese medicine, bu-zhong-yi-qi-tang (Japanese name: Hochu-ekki-to) on the protection against Listeria monocytogenes infection in mice. Immunopharmacol Immunotoxicol 1992;14:383–402.
11. Hossain MS, Takimoto H, Hamano S, Yoshida H, Ninomiya T, Minamishima Y, et al. Protective effects of Hochu-ekki-to, a Chinese traditional herbal medicine against murine cytomegalovirus infection. Immunopharmacology 1999;41:169–81.
12. Mori K, Kido T, Daikuhara H, Sakakibara I, sakata T, Shimizu K, et al. Effect of Hochu-ekki-to (TJ-41): a Japanese herbal medicine, on the survival of mice infected with influenza virus. Antiviral Res 1999;44:103–11.
13. Abe S, Tansho S, Ishibashi H, Akagawa G, Komatsu Y, Yamaguchi H. Protection of immunosuppressed mice from lethal Candida infection by oral administration of a kampo medicine, Hochu-ekki-to. Immunopharmacol Immunotoxicol 1999;21: 331–42.
14. Utsuyama M, Seidlar H, Kitagawa M, Hirokawa K. Immunological restoration and anti-tumor effect by Japanese herbal medicine in aged mice. Mech Ageing Dev 2001;122:341–52.
15. Saiki I. A Kampo medicine "Juzen-taiho-to" prevention of malignant progression and metastasis of tumor cells and the mechanism of action. Biol Pharm Bull 2000;23:677–88.
16. Cho JM, Sato N, Kikuchi K. Prophylactic anti-tumor effect of Hochu-ekki-to (TJ41) by enhancing natural killer cell activity. In Vivo 1991;5:389–91.
17. Ohtake N, Nakai Y, Yamamoto M, Sakakibara I, Takeda S, Amagaya S, et al. Separation and isolation methods for analysis of the active principles of Sho-saiko-to (SST) oriental medicine. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812; 135–48.
18. Iwata S, Taki Y, Kawai Y, Kanai M, Takabayashi A. Mitochondrial membrane potential is reduced in peripheral natural killer cells following partial hepatectomy. Immunol Lett 2002;82: 225–33.
19. Rottenberg H, Wu S. Quantitative assay by flow cytometry of the mitochondrial membrane potential in intact cells. Biochem Biophys Acta 1998;1404;393–404.
20. Pross HF, Baines MG. Spontaneous human lymphocyte-mediated cytotoxicity against target cell. I: The effect of malignant disease. Int J Cancer 1976;18:593–601.
21. Bryant J, Day R, Whiteside TL, Herberman RB. Calculation of lytic units for the expression of cell-mediated cytotoxicity. J Immunol Methods 1992;146:91–103.
22. Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM. In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci 2000;97:2731–6.
23. Tönnesen E, Wahlgreen C. Influence of extradural and general anaesthesia on natural killer cell activity and lymphocyte subpopulations in patients undergoing hysterectomy. Br J Anaesth 1988;60:500–7.
24. Tanabe H. Study of effect of surgical stress on immunity in patients with gastrointestinal cancer. Arch Jpn Chir 1993;62: 145–52.
25. Vallejo R, Hord ED, Barna SA, Santiago-Palma J, Ahmed S. Perioperative immunosuppression in cancer patients. J Environ Pathol Toxicol Oncol 2003;22:139–46.
26. Li T, Tamada K, Abe K, Tada H, Onoe Y, Tatsugami K, et al. The restoration of the antitumor T cell response from stress-induced suppression using a traditional Chinese herbal medicine Hochu-ekki-to (TJ-41:Bu-Zhong-Yi-Qi-Tang). Immunopharmacology 1999;43:11–21.
27. Kuroiwa A, Liou S, Yan H, Eshita A, Naitoh S, Nagayama A. An effect of a traditional Japanese herbal medicine: Hochu-ekki-to (Bu-Zhong-Yi-Qi Tang), on immunity in elderly persons. Int Immunopharmacol 2004;4:317–24.
28. Weissman C. The metabolic response to stress: an overview and update. Anesthesiology 1990;73:308–27.
29. Blalock JE. The syntax of immune-neuroendocrine communication. Immunol Today 1994;15:504–11.