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Prospective Study for Korean Red Ginseng Extract as an Immune Modulator following a Curative Surgery in Patients with Advanced Colon Cancer

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Abstract : In this paper, we present evidence that the Korean red ginseng extract shows the immunomodulatory activities during postoperative chemotherapy after curative surgery in patients with advanced colon cancer. We measured the circulating interleukin-2 (IL-2), interleukin-8 (IL-8)and interleukin-10 (IL-10) as a immune modulator to evaluate the effect of Korean red ginseng. The mean preoperative value of IL-2 was similar in the non-RG group and the RG group (5.72 pg/ml versus 6.87 pg/ml, $p>0.05$). The mean value of IL-2 was compared with IL-2 from healthy control group, there was no significant difference (14.89 pg/ml versus 14.22 pg/ml, $p>0.05$). The mean preoperative value of IL-8 was higher in the non-RG group comparing with the RG group (30.92 pg/ml versus 36.25 pg/ml, $p < 0.05$). At postoperative 3 month, the mean values of IL-8 from non-RG and RG group down to 24.56 pg/ml and 21.46 pg/ml respectively. The IL-8 of RG group at 3 month showed no difference with that of HC group(21.46 pg/ml versus 16.31 pg/ml, $p>0.05$). The preoperative mean value of IL-10 of non-RG, RG and HC group was 11.56 pg/ml, 10.8 pg/ml, and 3.68 pg/ml respectively. At postoperative 3 month, the mean values of IL-10 from non-RG and RG group down to 8.45 pg/ml and 5.04 pg/ml respectively. In spite of decreasing IL-10 levels of both cancer patients group with time, there was still significant difference with that of HC group (non-RG versus HC group, $p=0.00$, RG versus HC group, $p=0.04$). The results of this study suggest that the red ginseng extract may have some immunomodulatory properties associated with IL-2, IL-8 and IL-10 activity in patients with advanced colorectal cancer during postoperative chemotherapy. We think to need the further studies and a larger sample size to fully evaluate the antitumor effect of ginseng and need to establish this mechanism of action as well as identify the active components associated with antitumor activity and immunomodulation in patients with advanced colorectal cancer.

Key words : Korean Red Ginseng, colon cancer, interleukins, anticancer

INTRODUCTION

The red ginseng has been reported to help increase physical endurance and stimulate physical and mental performance as an East Asian traditional herbal medicine.[1] The red ginseng extract is made by steamed and sundried six-year-old Korean ginseng root, leaving the epidermis intact, yielding a product with an aromatic fragrance, bitter sweet taste, and yellowish brown to dark brown color. In Korea, it is a common belief that the red ginseng may augment conventional cancer treatment and it is frequently used as a complementary drug for cancer after operation. The red ginseng and some extracts of Panax ginseng showed anticarcinogenic actions including inhibition of tumor angiogenesis and metastasis[2-4] and induction of apoptosis in tumor cells.[5] Another study has shown that an extract from Panax ginseng is able to activate multiple effector pathways of immunostimulation for anti-tumor action.[6] Colon cancer is a forth leading cause of cancer-related mortality in Korea. Despite varied treatment strategies, the control of the colon cancer with an advanced stage remains yet problematic. The aim of this study was to prospectively evaluate the effect of the red ginseng on postoperative host immunity l in patients of advanced colon cancer. The authors prospectively investigated the circulating interleukin-2 (IL-2), Interleukin-8 (IL-8), Interleukin-10 (IL-10) according to ingestion of the red ginseng in patients with advanced colon cancer who had underwent the curative resection with postoperative chemotherapy.

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SUBJECTS AND METHOD

Patient Characteristics

Forty-seven patients who had undergone the curative colon resection by the same surgeon for histolopathologically proven AJCC stage III colon adenocarcinoma were considered eligible for this study. The patients were identified preoperatively between July 1, 2005 and February 30, 2006. Datas regarding clinical characteristics were collected and evaluated by a blinded, independent surgeon. Patients were excluded from this study if they had: (1) perioperative or postoperative transfusion required during follow-up, (2) postoperative major complications such as single or multiple organ failure, sepsis, pneumonia, anastomosis leakage, intrabdominal infection or abscess, (3) reoperation, (4) failure to maintain the ingestion of the red ginseng extract, (5) failure in regular follow-up, or (6) death unrelated to primary cancer, or (7) chronic medical illness such as hypertension, diabetes mellitus, and chronic obstructive pulmonary diseases, etc. After exclusion criteria, 47 patients were eligible and enrolled in this study. All patients were treated with 5-Fu +leukovorin chemotherapy every month during the first six months after the curative operation. 5-FU and leukovorin (375 mg/m^2/day and 30 mg IV/bolus/day) regimen was administered by continuous infusion. All patients were randomly allocated into two groups before operation: patients designated to receive the red ginseng (RG group, n = 24) and control group (non-RG group, n = 23). The RG group took the red ginseng extract (Korea Ginseng Corporation, Seoul, Korea) orally at a dosage of 3 g/day during the first three months after operation. IL-2, IL-8 and IL-10 were evaluated at preoperative day and postoperative months 1 and 3 from peripheral venous blood draws. This study was performed after informed consent was obtained.

ELISA for IL-2, IL-8 and IL-10

The blood sample was collected at preoperative 1 day, postoperative 1 month, and 3 month. Blood samples from each group(non-RG, RG and HC group) were extracted in 100 μl of PBS containing the protease inhibitor cocktail (Complete, Roche Diagnostics) and sonicated at 10 watts (BRANSON SONIFIER 250, Danbury, CT) for 5 s on ice. The supernatant was collected following centrifugation at 14,000 g for 10 min at 4°C (Micromax RF, IEC, Needham heights, MA). Two hundred μg of serum was used for ELISA (Biosource, Camarillo, CA), performed according to the manufacturer's guidelines. The absorbance at 450 nm wavelength was measured using a 96 well plate by spectrophotometry (Spectramax 190, Molecular decevice, Sunnyvale CA).

RESULTS

No adverse effect was observed in both groups during the observation period. The clinical and histopathological details of all patients with AJCC stage III colon cancer studied are shown in Table 1. There were no significant statistical differences between the RG and non-RG groups based on age, sex ratio, AJCC stage, depth of tumor invasion, regional lymph node metastasis, tumor size, proximal resection margin and distal resection margin.

The mean preoperative value of IL-2 was similar in the non-RG group and the RG group (5.72 pg/ml versus 6.87 pg/ml, $p>0.05$). At 1 month, the mean value of IL-2 in RG group increased slightly upto 8.83 pg/ml, but there was no significance comparing to non-RG group. At 3 month, the

Table 1. The clinicopathological characteristics of colon cancer patients.

Variables	non-RG group* (n=24)	RG group† (n=23)	p value
age (years)	63.7±10.2	65.6±8.9	NS‡
gender			NS
male	13	11	
female	11	12	
depth of invasion			NS
T1	2	1	
T2	5	3	
T3	15	19	
T4	2	0	
LN metastasis			NS
N0	12	14	
N1	8	7	
N2	4	2	
distant metastasis			NS
M0	24	23	
M1	0	0	
tumor size (cm)	4.7±2.2	5.7±2.3	NS
PRM§ (cm)	15.2±10.2	13.7±10.3	NS
DRM‖ (cm)	9.1±10.3	7.5±7.0	NS

*non-RG group: patients not given the red ginseng extract during chemotherapy
†RG group: patients given the red ginseng extract during chemotherapy
‡NS: not significant
§PRM: proximal resection margin
‖DRM: distal resection margin
Plus-minus values are standard deviations.

Table 2. Comparison of mean value of interleukin according to ginseng treatment and time interval.

	Group	Mean value (pg/ml)	*p*-value		Group	Mean value (pg/ml)	*p*-value		Group	Mean value (pg/ml)	*p*-value
IL-2 (preop*)	non-RG§	5.72	0.302	IL-2 (preop)	non-RG	5.72	0.00	IL-2 (preop)	RG	6.87	0.00
	RG‖	6.87			HC¶	14.89			HC	16.31	
IL-2 (1 m†)	non-RG	7.22	0.156	IL-2 (1 m)	non-RG	9.92	0.00	IL-2 (1 m)	RG	8.83	0.00
	RG	8.83			HC	14.89			HC	16.31	
IL-2 (3 m‡)	non-RG	9.92	0.000	IL-2 (3 m)	non-RG	9.92	0.00	IL-2 (3 m))	RG	14.22	0.628
	RG	14.22			HC	14.89			HC	16.31	
IL-8 (preop)	non-RG	36.25	0.15	IL-8 (preop)	non-RG	36.25	0.00	IL-8 (preop)	RG	10.92	0.00
	RG	30.92			HC	16.31			HC	16.31	
IL-8 (1 m)	non-RG	26.82	0.254	IL-8 (1 m)	non-RG	26.82	0.04	IL-8 (1 m)	RG	23.44	0.25
	RG	23.44			HC	16.31			HC	16.31	
IL-2 (3 m)	non-RG	24.56	0.233	IL-2 (3 m)	non-RG	24.56	0.13	IL-2 (3 m)	RG	21.46	0.97
	RG	21.46			HC	16.31			HC	16.31	
IL-10 (preop)	non-RG	11.56	0.458	IL-10 (preop)	non-RG	11.56	0.00	IL-10 (preop)	RG	10.80	0.00
	RG	10.80			HC	3.68			HC	3.68	
IL-10 (1 m)	non-RG	8.69	0.004	IL-10 (1 m)	non-RG	8.69	0.00	IL-10 (1 m)	RG	6.17	0.00
	RG	6.17			HC	3.68			HC	3.68	
IL-10 (3 m)	non-RG	8.45	0.002	IL-10 (3 m)	non-RG	8.45	0.00	IL-10 (3 m)	RG	5.04	0.04
	RG	5.04			HC	3.68			HC	3.68	

*preop: preoperative value, †1 m: postoperative 1 month value, ‡3 m: postoperative 3 month value, §non-RG: patients not given red ginseng extract during chemotherapy ‖RG: patients given red ginseng extract during chemotherapy, ¶HC: healthy control group

mean value of IL-2 increased upto 14.22 pg/ml, and it was higher than non-RG group with statistical significance (14.22 pg/ml versus 9.92 pg/ml, $p<0.05$). And when the mean value of IL-2 was compared with IL-2 from healthy control group, there was no significant difference (14.89 pg/ml versus 14.22 pg/ml, $p>0.05$). (Table 2)

The mean preoperative value of IL-8 was higher in the non-RG group comparing with the RG group (30.92 pg/ml versus 36.25 pg/ml, $p<0.05$). But the mean values of IL-8 from non-RG group and RG group were significantly higher than HC group(30.92 pg/ml versus 16.31 pg/ml, 36.25 pg/ml versus 16.31 pg/ml, $p>0.05$). The mean value of IL-8 of cancer group, non-RG and RG group, deceased with time.

At 1 month, the mean value of IL-8 in RG group decreased slightly at23.44 pg/ml, and that of non-RG group at 26. 82 pg/ml, and there was no significance between two group. When it compared with the mean value of HC group, the mean value of IL-8 was still significantly higher in cancer patients. At postoperative 3 month, the mean values of IL-8 from non-RG and RG group down to 24.56 pg/ml and 21.46 pg/ml respectively. The IL-8 of RG group at 3 month showed no difference with that of HC group(21.46 pg/ml versus 16.31 pg/ml, $p>0.05$), but that of non-RG group was higher than HC group($p=0.013$) (Table 2).

Preoperative mean value of IL-10 of non-RG, RG and HC group was 11.56 pg/ml, 10.8 pg/ml, and 3.68 pg/ml respectively. Preoperative IL-10 levels of cancer patients were significantly higher than that of healthy volunteer. At 1 month, the mean value of IL-10 in RG group decreased slightly at 6.17 pg/ml, and that of non-RG group at 8.69 pg/ml, and there was no significance between two group. When it compared with the mean value of HC group, the mean value of IL-10 was still significantly higher in cancer patients. The value of IL-10 decreased with time by three month. At postoperative 3 month, the mean values of IL-10 from non-RG and RG group down to 8.45 pg/ml and 5.04 pg/ml respectively. The mean value of IL-10 at three month of RG group decreased lesser than that of non-RG group($p<0.05$). In spite of decreasing IL-10 levels of both cancer patients group, there was still significant difference with that of HC group (non-RG versus HC group, $p=0.00$, RG versus HC group, $p=0.04$) (Table 2).

DISCUSSION

Although we do not completely understand the mechanisms that underlie the specific immunologic alterations, it is clear that both functional and quantitative defects in immunity develop with cancer, especially in advanced

stages.[7] Indeed, a number of studies have reported that in the immune status and prognosis of patients with solid neoplasms, there is a reduction in IL-2 value of peripheral circuating blood, and increased IL-8 and IL-10 that are thought to play an important role in cell-mediated immunity. In the present study, the preoperative values of IL-2, IL-8 and IL-10 were similar to the results of other studies of gastric cancer patients from Korea.[21]

The species of ginseng used in the present study was Korean red ginseng, which is cultivated in Korea. In a prospective case control study from Korea, it was shown that the risk of all kinds of cancers, including gastric cancer, was decreased by red ginseng intake in a dose-dependent manner.[8] Although it is still unknown which components of ginseng are able to reduce the risk of cancers, the preventive effect of ginseng against cancers was observed in almost all types of ginseng products, which included fresh ginseng extract, white ginseng extract and powder, and red ginseng products.[9] Ginseng saponins (ginsenosides) have been regarded as the principal components responsible for the pharmacological activities of ginseng.[5] The ginsenosides, such as Rb1, Rb2, Rc and Rg have been reported to have antitumor effects, particularly on the inhibition of tumor-induced angiogenesis, tumor invasion and metastasis, and the control of phenotypic expression and differentiation of tumor cells in vivo and in vitro.[2,5,10-13] However, the mechanisms of antitumor effects of ginsenosides are yet to be understood completely.

IL-2 is known to activate antitumor activities. It activate lymphokine activated killer(LAK) cells and also have synergy with LAK cells. IL-2 level is observed to be decreased in the advanced cancer. In our study the preoperative IL-2 levels of colorectal carcinoma patients were decreased.

The cell-mediated cytotoxicity was depressed in patients with advanced gastric cancer, especially stage IV gastric cancer. It was induced by the decreased production of serum Interleukin-2 (IL-2) that was recognized as a T-cell growth factor.[14, 15] The gastric cancer cells produced serum immunosuppressive factors that suppressed antitumor cytotoxicity of IL-2-induced lymphocytes.[16] Furthermore, the incidence of lymph node metastasis was increased by the functional deficit of T lymphocytes and the elevated CD8. These were also induced by the reduced production of serum IL-2 in patients with advanced gastric cancer.[17, 21] The extract of Panax ginseng (ginsan) inhibited pulmonary metastasis of melanoma cells, induced macrophage cytokines, and generated lymphokine-activated killer (LAK) cells in synergy with IL-2.[18] However, the antitum or cytotoxicity of the red ginseng remains unclear in gastric cancer. Similar observation was described in colorectal carcinoma.

Interleukin-8 (IL-8) is a member of the chemokine family of pro-inflammatory chemotactic cytokines and is secreted by some human colorectal carcinoma cell lines. When IL-8 mRNA was detected within the cytoplasm of tumour cells in all nine samples tested, including that of a tumour which had metastasised to a lymph node. IL-8 protein was detected in tumour samples and was mainly localised to the tumour cell cytoplasm. Some infiltrating leucocytes, endothelial cells and fibroblast-like cells within the tumour sections were also positive for IL-8 mRNA and protein. So there is possibilities that colorectal tumours produce IL-8 to aid invasion and/or metastasis or as a tumour growth factor.[19] In our study, the IL-8 level of carcinoma patients level were increased higher than HC group by 2.25 times grossly. The level was deceased with the time but it did not come to normal level at postoperative 3 month. But the level of IL-8 of RG group gradually approached to the mean value of IL-8 of HC group at 3 month later in contrast to that of non-RG group still had larger gap with HC group.

IL-10 is a Th2 anti-inflammatory cytokine that plays a crucial role in modulating gastrointestinal tract inflammation. The IL-10–deficient mouse develops atrophic gastritis, chronic enterocolitis, gastric cancer[22] and ultimately colorectal cancer[20].

IL-10 was also decreased with the time and ware increasing highe than gastric cancer which was reported the prospective study for Korean red ginseng extract as an immune modulator followin curative gastric resectin in advanced gastric cancer.[21,22] In this study, the IL-8 level of carcinoma patients level were increased higher than HC group. The level was deceased with the time and but it came to normal level at postoperative 3 month in RG group, but still remains higher than HC group in non-RG group.

The administrated dose of the red ginseng extract was 3 g/day in our study for the following reason: the empirical dose pulverized red ginseng in traditional herbal medicine is 3-6 g/day. We did not perform a phase I escalating dose study. The present study is the first clinical prospective study evaluating the effects of the red ginseng for advanced colon cancer, although the number of patients who were enrolled in this study was small to fully evaluate the therapeutic effects of ginseng on postoperative host immunity and survival. However, in spite of this lim-

itation, it suggests that the red ginseng extract may help to improve the postoperative survival in patients with advanced cancer.

Despite this limitation,these results suggest that postoperative intake of the Korean red ginseng extract have potential to improve earler anticancer immunity with recovering IL-2 and reducing IL-8 and 10 from the depressed IL-2 and elevated IL-8 and 10 by colon cancer patients during postoperative chemotherapy. But further studies need a larger sample size to fully evaluate the antitumor effect of ginseng and need to establish this mechanism of action as well as identify the active components associated with antitumor activity and immunomodulation in patients with advanced colon cancer.

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REFERENCES

1. Chong, S.K. and Oberholzer, V.G.: Ginseng-Is there a use in clinical medicine? *Postgrad. Med. J.* **64**, 841-846 (1988).
2. Mochizuki, M., Yoo, Y.C., Matsuzawa, K., Sato, K., Saiki, I., Tono-oka, S., Samukawa, K. and Azuma, I.: Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)- and 20(S)-ginsenoside-Rg3, of red ginseng. *Biol. Pharm. Bull.* **18**, 1197-1202 (1995).
3. Sato, K., Mochizuki, M., Saiki, I., Yoo, Y.C., Samukawa, K. and Azuma, I.: Inhibition of tumor angiogenesis and metastasis by a saponin of Panax ginseng, ginsenoside-Rb2. *Biol. Pharm. Bull.* **17**, 635-639 (1995).
4. Xiaoguang, C., Hongyan, L., Xiaohong, L., Zhaodi, F., Yan, L., Lihua, T., Rui, H.: Cancer chemopreventive and therapeutic activities of red ginseng. *J. Ethnopharmacol.* **60**, 71-78 (1998).
5. Wakabayashi, C., Murakami, K., Hasegawa, H., Murata, J. and Saiki, I.: An intestinal bacterial metabolite of ginseng protopannaxadiol saponins has the ability to induce apoptosis in tumor cells. *Biol. Pharm. Bull.* **246**, 725-730 (1998).
6. Lee, Y.S., Chung, I.S., Lee, I.R., Kim, K.H., Hong, W.S. and Yun, Y.S.: Activation of multiple effector pathways of immune system by the antineoplastic immunostimulator acidic polysaccharide ginsan isolated from Panax ginseng. *Anticancer Res.* **17**, 323-331 (1997).
7. Kaszubowski, P.A., Husby, G., Tung, K.S.K. and Williams, R.C. Jr.: T-lymphocyte subpopulations in peripheral blood and tissues of cancer patients. *Cancer Res.* **40**, 4648-4657 (1980).
8. Yun, T.K. and Choi, S.Y.: Non-organ specific cancer prevention of ginseng: a prospective study in Korea. *Int. J. Epidemiol.* **27**, 359-364 (1998).
9. Yun, T.K. and Choi, S.Y.: Preventive effect of ginseng intake against various human cancers: a casecontrol study on 1987 pairs. *Cancer Epidemiol. Biomarkers Prev.* **4**, 401-408 (1995).
10. Iishi, H., Tatsuta, M., Baba, M., Uehara, H., Nakaizumi, A., Shinkai, K., Akedo, H., Funai, H., Ishiguro, S. and Kitagawa, I.: Inhibition by ginsenoside Rg3 of bombesin-enhanced peritoneal metastasis of intestinal adenocarcinomas induced by azoxymethane in Wistar rats. *Clin. Exp. Metastasis* **15**, 603-611 (1997).
11. Lee, Y.J., Chung, E., Lee, K.Y., Lee, Y.H., Huh, B. and Lee, S.K.: Ginsenoside-Rg1, one of the major active molecules from Panax ginseng, is a functional ligand of glucocorticoid receptor. *Mol. Cell. Endocrinol.* **20**, 135-140 (1997).
12. Matsunaga, H., Katano, M., Yamamoto, H., Mori, M. and Takata, K.: Studies on the panaxytriol of Panax ginseng C. A. Meyer. Isolation, determination and antitumor activity. *Chem. Pharm. Bull.* **37**, 1279-1281 (1989).
13. Odashima, S., Nakayabu, Y., Honjo, N., Abe, H. and Arichi, S.: Induction of phenotypic reverse transformation by ginsenosides in cultured Morris hepatoma cells. *Eur. J. Cancer* **15**, 885-892 (1979).
14. Akiyoshi, T., Koba, F., Arinaga, S. and Ueo, H.: Preoperative cell-mediated immune function and the prognosis of patients with gastric carcinoma. *J. Surg. Oncol.* **45**, 137-142 (1990).
15. Suminami, Y., Kashii, Y., Law, J.C., Lin, W.C., Stanson, J., Reichert, T.E., Rabinowich, H. and Whiteside, T.L.: Molecular analysis of the IL-2 receptor beta chain gene expressed in human tumor cells. *Oncogene* **16**, 1309-1317 (1998).
16. Sugiyama, Y., Sakata, K., Saji, S., Takao, H. and Hamuro, J.: Effects of serum immunosuppressive factors on antitumor cytotoxicity of interleukin 2-induced lymphocytes. *J. Surg. Oncol.* **35**, 223-229 (1987).
17. Tsubono, M., Nio, Y., Shiraishi, T., Morimoto, H., Tseng, C.C., Kawabata, K., Masai, Y., Fukumoto, M. and Tobe, T.: Increased number of suppressor T-cells and impaired IL-2 mediated T-cell function in peripheral blood of gastric cancer patients. *J. Clin. Lab. Immunol.* **33**, 107-115 (1990).
18. Kim, K.H., Lee, Y.S., Jung, I.S., Park, S.Y., Chung, H.Y., Lee, I.R. and Yun, Y.S.: Acidic polysaccahride from Panax ginseng, ginsan, induces Th1 cell and macrophage cytokines and generates LAK cells in synergy with rIL-2. *Planta Med* **64**, 110-115 (1998).
19. Brew, R., Southern, S.A., Flanagan, B.F., McDicken, I.W. and Christmas, S.E.: Detection of interleukin-8 mRNA and protein in human colorectal carcinoma cells. *Eur J Cancer* **32A**(12), 2142-2147 (1996).

20. Evans, C., Morrison, I., Heriot, A.G., Bartlett, J.B., Finlayson, C., Dalgleish, A.G. and Kumar, D.: The correlation between colorectal cancer rates of proliferation and apoptosis and systemic cytokine levels; plus their influence upon survival. *Br J Cancer* **94**(10), 1412-1419 (2006).
21. Sung Ock Suh, Jin Kim and Ming Young Cho.: The prospective Study for Korean Red Ginseng Extract as an Immune Modulator following a Curative Gastric Resection with Advanced Gastric Cancer. *J. Ginseng Res.* 28 (2004).
22. Sung Ock Suh, Matthew Koh, Nam Ryeol Kim and Ming Young Cho Effect of Red Ginseng uopn Postoperative Immunity and Survival in Patients with Stage III gastric Cancer. *The Amercan J. of Chinese Med.* **30**, 483-494 (2002).