J Korean Med Sci 2001; 16(Suppl): S70-4
ISSN 1011-8934



# Study on Chemoprevention of Hepatocellular Carcinoma by Ginseng: An Introduction to the Protocol

In patients with chronic hepatitis C virus disease, there is a high incidence of development of hepatocedllular carcinaoma (HCC) in the process of transition from chronic hepatitis to hepatic cirrhosis. Although ginseng traditionally has been used mainly as a nutritional supplement in Asian countries, a case-control study found that it may inhibit the development of HCC. We therefore planned a clinical study of HCC prevention by medicinal ginseng. The subjects are patients with chronic C virus disease (chronic hepatitis and hepatic cirrhosis), who are high risk group for HCC. This intervention study is a multi-center, double-blind, randomized controlled trial. The participants will be randomly divided into two groups. The test sample (1 g of red ginseng powder per day) will be administered for 5 yr, and ginseng intake will be prohibited during the administration period. The primary endpoint of this study is the development of HCC. Target number of recruiting subjects are 300. The participants should be registered from February 2001 to January 2003.

Key Words : *Chemoprevention; Carcinoma, Hepatocellular; Ginseng*

**The Ginseng-HCC Chemopreventive Study Osaka Group**

The members of the Ginseng-HCC Chemopreventive Study Osaka Group are listed in the appendix.

**Address for correspondence**
Hideki Ishikawa
Department of Cancer Epidemiology, Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases. 3-3 Nakamichi 1-Chome, Higashinari-Ku, Osaka 537-8511, Japan
Tel : +81.6-6972-1181, Fax : +81.6-6981-3000
E-mail : cancer@gol.com

## INTRODUCTION

The risks of hepatocellular carcinoma (HCC) can increase due to the progression of chronic hepatitis to hepatic cirrhosis in patients with chronic hepatitis C virus (HCV) disease (1). The 5-yr follow-up study revealed that the incidence of HCC was approximately 37% among the HCV associated cirrhosis patients (2). In spite of the development of advanced therapies including transcatheter arterial embolization and percutaneous ethanol injection, high recurrence and poor prognosis are frequently observed in HCC patients. According to the Osaka Cancer Registry, Japan, in 1987 the 5-yr relative survival rate of HCC patients was considerably low (5.5%). Thus, in order to improve the prognosis of chronic HCV disease patients, who are a high-risk group of HCC, the prevention of HCC development is particularly important as well as periodical check-ups for early detection.

For thousands of years, ginseng has been used as a tonic and nutrient in Asian countries. Ginseng has been shown to improve one's general condition and non-specific complaints due to exhaustive and feverish illnesses through enhancement of one s natural healing power. Also, ginseng has been shown to inhibit carcinogenesis in animal models (3, 4). Nishino (5) found that ginseng significantly inhibited the development of spontaneous liver tumors in C3H/He male mice. A recent case-control study in Korea (6, 7) demonstrated the prevention of cancer development in several organs by ginseng ingestion.

Ginseng is formulated in a variety of medicines and Sho-saiko-to is one of the well-known Kampo medicines that contains ginseng. The intervention study using Sho-saiko-to suggested that it could prevent the development of HCC in chronic hepatitis patients (8). Regrettably, this medicine has been reported to cause a severe side effect, interstitial pneumonia, however, it is believed that ginseng did not contributes to this side effect. Therefore, we planned a multi-center, double-blind, randomized controlled trial to determine the preventive effect of ginseng on HCC in chronic HCV disease patients who are a high risk group of HCC.

## MATERIALS AND METHODS

This intervention study will be conducted at 8 hospitals including Toyonaka Municipal Hospital, Izumiotu Municipal Hospital, Izumisano Municipal Hospital, Suita Municipal Hospital, Kawanishi City General Hospital, Otemae Hospital, Ikeda Municipal Hospital and National Shikoku Cancer Center.

### Inclusion Criteria

The subjects should be chronic HCV disease patients, such as those with chronic hepatitis and cirrhosis, and be negative for hepatitis B surface antigen and positive for HCV antibody or HCV-RNA. L-aspartate:2-oxaloglutarate amino-

transferase (AST) and L-alanine:2-oxoglutarate aminotranferase (ALT) in the chronic hepatitis patients should be out of the normal range. In hepatic cirrhosis patients, ALT and AST should be out of the normal range or conclusively diagnosed for hepatic cirrhosis with an abdominal ultrasonograph or histological examination. In the chronic hepatitis patients who were administered with interferon, disappearance of virus and normalization of ALT and AST should not be observed during the one-year follow-up after the interferon treatment. The patients who are not treated with interferon can be enrolled, however, the precise explanation as to why the patients are not on interferon therapy should be noted. Subjects who receive interferon therapy after enrolling in this study should be excluded as a dropout case. Hepatic cirrhosis patients, classified as being in Compensated hepatic cirrhosis (Child A), can be eligible for the study. After a full explanation of the details of the study in writing, patients over 40 who have signed the Agreement can be eligible.

### Exclusion Criteria

Patients with the following criteria will be excluded: 1) those with a platelet count greater than 150 thousand/$\mu$L, 2) those who may be re-administered interferon, 3) those allergic to medicines and/or herbs, 4) current ginseng users who will not discontinue its use, 5) those with a history of malignant neoplasms or current cancer patients, 6) those who will be unable to comply with periodical follow-ups, including an abdominal ultrasonography, and 7) those who are one-time participants of this study.

### Recruiting and entry procedures, and sample administration

This intervention study is a multi-center, double-blind, randomized placebo-controlled trial. When collaborator-physicians examine patients who meet the inclusion criteria, they will ask them to participate in this study. After agreeing to participate in the study, the patients will sign the Agreement (informed consent). The physicians will fill out the "Case Card", and mail it to the secretariat of the study (Osaka Medical Center for Cancer and Cardiovascular Diseases). As soon as the secretariat receives the consent forms, they will register the patient as a participant.

The participants will be randomly divided into two groups: red ginseng administered group (GI group) and non-functional food administered group (NF group), using the categorized Block-Random methods according to the progression of chronic hepatitis (history of interferon therapy, and hepatic cirrhosis) and hospitals where the participants are recruited. A secretary will register the assignment of participants and inform Wakunaga Pharmaceutical Co., Ltd. A co-researcher of Wakunaga Pharmaceutical Co., Ltd. will send a test sample and a diary for recording sample ingestion to each participant every 3 months. The test sample will be administered for 5 yr, and ginseng intake will be prohibited during the administration period.

### Endpoint and examinations

The primary endpoint of this study is the development of HCC. An abdominal ultrasonography will be performed before and after starting the ingestion of the samples, and every 6 months during the intervention. An abdominal dynamic computed tomography will also be performed before and after starting the ingestion of the samples, and once every year during the intervention. Hepatitis C virus markers, hematological tests, biochemical tests, hemostasis and coagulation tests, serum $\alpha$-fetoprotein and serum PIVKA-II will be regularly determined at a certain period.

In order to confirm compliance, the participants should record daily the ingested capsule number on a designated form, and the bottle containing a 3-month supply of test samples will be collected after its use. Once a year, 20 participants will be randomly selected from each group and their urine will be collected and urinary content of 20(S)-protopanaxatriol, an aglycone of a major saponin in red ginseng, will be determined using GC-MS.

### Ethics committees

This study was approved by the following ethics committees: "the Ethics and Data Monitoring Committee" for this study, the ethics committee of Osaka Medical Center for Cancer and Cardiovascular Diseases, wherein the secretariat of the study is set, and the ethics committee of each hospital wherein the subjects will be recruited.

### Target number of recruiting subjects

Three hundred participants (150 per group) will be recruited for this study and the number of participants should not be less than 200. The number of participants for determining the preventive impacts of red ginseng on HCC will be evaluated based on the following. The annual incidence of HCC could be 3.5% in chronic hepatitis patients whom the interferon treatment was not successful and 6% in hepatic cirrhosis patients, respectively. If the same number of chronic hepatitis patients and hepatic cirrhosis patients participate in this study, the average incidence of HCC would be 4.75%. According to the case-control studies conducted by Yun, cancer preventive efficacy of ginseng was odds ratio (OR)=0.56 in the 1990's study (6), and OR =0.50 in total and OR=0.48 in the liver in the 1995's study (7). Furthermore, Nishino (5) reported that ginseng inhibited the development of spontaneous liver tumors in C3H/He male mice at 0.31. Thus, we estimated that the preventive effect of ginseng would be in the hazard ratio of 0.3 to 0.5. On assump-

tion of the power=80%, 100 to 150 participants per group are required in order to obtain a significant effect of a $p$ value less than 0.05 so that the minimum number of participants cannot be less than 100 in each group and is desirably 150. The participants should be registered from February 2001 to January 2003.

### Evaluation methods

The date of HCC development will be settled on the day when HCC is first detected by a physician through an abdominal ultrasonography or an abdominal dynamic computed tomography. The individual prevention period of HCC in participants will be determined between the date of assignment and the date of HCC development. In participants without detectable HCC development after 5 yr from the assigning date, their participation in this study will be censored at the end of the follow-up. If the participants die of another disease or by accident, their participation will be closed at the date of death. Participants who cannot be followed up will be managed as dropout cases.

The preventive effects of red ginseng on HCC will be primarily evaluated from the prevention period obtained from all registered participants using the intent-to-treat principle. Also, the prevention period obtained from participants who meet all requirements and have good compliance records will be evaluated.

The participants will be classified into two categories, chronic hepatitis and liver cirrhosis, and the cumulative incidence of HCC development in both categories will be separately estimated using the Kaplan-Meier methods. The estimated cumulative incidences will be analyzed using the staratified Logrank test (two sided). If unbalances of prognostic factors were seen between treatment groups, the stratified proportional hazard models will be used to adjust the effect of confounding. The potential confounding factors will be determined through the background knowledge and preliminary analyses. The effect measure (Hazard ratio) of ginseng on HCC prevention will be estimated as the point estimate with 90 and 95% confidence intervals. The validity of the assumption of the proportional hazard models will be checked by using graphical approaches or time-dependent covariates.

The interim analysis will be conducted at one and three years after the completion of registration. If the Ethics Committee of this study concludes that the ginseng treatment is significantly effective or that no efficacy would be expected for further continuation of the study after examining the interim analysis, the Ethics and Data Monitoring Committee will recommend termination of the study.

### Test Sample

The samples will be provided by Wakunaga Pharmaceutical Co., Ltd. (Hiroshima, Japan). Each capsule for the GI group will contain 250 mg of the powdered red ginseng processed from the roots of *Panax ginseng* C.A. Meyer cultivated in China and 10 mg of sucrose esters of fatty acids, and 4 capsules (1 g of red ginseng powder) will be ingested daily. Each capsule for the NF group will contain 250 mg of corn starch and 10 mg of sucrose esters of fatty acids, and 4 capsules will be ingested daily.

### Current Status

The first protocol was submitted to the Ethics Committee for this study in March, 2000, and the Committee directed to amend the protocol after their consideration. The revised protocol was submitted to the Committee November, 2000, and approved. Then, the protocol was submitted to the ethics committee of each hospital for their approval. Up through March, 2001, three ethics committees approved the study, and the collaborator-physicians are now recruiting patients at those hospitals.

## DISCUSSION

The follow-up study showed that the incidence of HCC was approximately 37% for 5 yr in HCV associated cirrhosis patients. According to the Osaka Cancer Registry, Japan, the 5-yr relative survival rate of HCC patients in 1987 was 5.5% and their prognosis was extremely poor. The development of procedures to prevent HCC is urgently desired.

Recently, interferon has been shown to successfully prevent the development of HCC9 (10). However, hepatitis C virus could not be eliminated by interferon therapy in 30% of the patients. Moreover, interferon therapy for liver cirrhosis patients is not covered by public insurance. This suggests that interferon therapy alone is not sufficient for HCC prevention and the methods to make up this therapy are in need. Sho-saiko-to is a well-known Kamo medicine containing ginseng and has been shown to inhibit liver cancer development (8). Unfortunately, Sho-saiko-to alone or in combination therapy with interferon was reported to cause a severe side effect, interstitial pneumonia. Interstitial pneumonia is an allergic disease and eventually leads to severe conditions. Allergic reactions occurring in the interstitium of the lung can be caused by medicines including Sho-saiko-to. The lymphocyte stimulation test frequently reveals positive reactions in patients to scutellaria root, bupleurum root and pinellia tuber, which are contained in Sho-saiko-to. Scutellaria root-containing Kampo medicines such as Saibokuto, Saireito and Rokkunshito are also reported to cause interstitial pneumonia. Thus, the interstitial pneumonia induced by Sho-saiko-to has been speculated to be mainly due to scutellaria root. However, since Bakumondoto, which does not contain scutellaria root has been shown to cause interstitial pneumonia, herbs other than scutellaria root also cause pneumonia. Gin-

seng occasionally shows a positive reaction in the lymphocyte stimulation test, however, it is unlikely to cause interstitial pneumonia since its historical and current uses have not resulted in pneumonia.

Ginseng has been shown to enhance natural healing power and to improve general conditions and non-specific complaints due to the exhaustive and feverish illness through enhancement of natural healing power. Also, ginseng has been shown to inhibit carcinogenesis in animal models (3-5). A recent case-control study in Korea demonstrated the prevention of cancer development in several organs by ginseng ingestion (6, 7).

According to these data, a cancer-preventive study for the high-risk group of HCC seems warranted. Thus, scientists of various endeavour, including epidemiologists, biochemists, pathologists, pharmacologists, bio-statisticians, physicians and a pharmaceutical manufacturer, have collaborated, and collected information regarding ginseng and cancer. After analyzing all the available informations, all members discussed in great detail the possible success of an intervention study. In the course of discussion, Fukushima and his colleagues who conducted animal carcinogenesis experiments, stated that ginseng did not increase or decrease the development of preneoplastic lesions in the liver. Nishino et al. found that red ginseng rather than white ginseng significantly inhibited the development of spontaneous liver tumors in mice. According to available data and our own studies, we concluded that the intervention trial of ginseng on HCC could be applicable to the high risk group. The pharmacokinetics and safety studies of red ginseng were conducted using healthy volunteers from January to February, 2000. No significant complaints or side effects due to red ginseng intake were reported, and the bio-compliance marker for red ginseng intake was established. After the completion of these steps, this multi-center, double-blind, randomized, placebo-controlled trial was designed, and is now in progress.

Researchers from various scientific fields have collaborated in the planning of this study. In the past, such a collaborative study has never been conducted in Japan. Thus, we sincerely expect good results from this study by the time of its completion within 7 yr.

## APPENDIX

The members of the Ginseng-HCC Chemopreventive Study Osaka Group are as follows: Hideki Ishikawa, Takaichiro Suzuki, Toru Otani (Osaka Medical Center for Cancer and Cardiovascular Diseases), Taik-Koo Yun (Korea Cancer Center Hospital), Hoyoku Nishino (Department of Biochemistry, Kyoto Prefectural University of Medicine), Sumio Kawata (Second Department of Internal Medicine Yamagata University School of Medicine), Shoji Fukushima (Department of Pathology Osaka City University Medical School), Yutaka Matsuyama (Department of Biostatistics Kyoto University School of Public Health), Osamu Tanaka (Institute of Pharmaceutical Sciences, Faculty of Medicine, Hiroshima University), Yasuharu Imai, Kazuto Fukuda (Department of Internal Medicine, Ikeda Municipal Hospital), Masami Inada, Yukihiko Matsuda (Department of Internal Medicine, Toyonaka Municipal Hospital), Iwao Yabuuchi (Department of Internal Medicine, Otemae Hospital), Shuzo Noda (Department of Internal Medicine, Suita Municipal Hospital), Shinji Tamura (Second Department of Internal Medicine, Osaka University Medical School), Yuichi Maeda (Department of Internal Medicine, Kawanishi City General Hospital), Yasuhiro Shirai (Department of Internal Medicine, Izumisano Municipal Hospital), Yoshirou Kayanoki (Department of Internal Medicine, Izumiotsu Municipal Hospital), Hiromichi Sumiyoshi, Toru Fuwa (Wakunaga Pharmaceutical Co., Ltd.), Shoji Shibata (Shibata Laboratory of Natural Medicinal Materials), Kenji Jinno (Clinical Division, National Shikoku Cancer Center). The members of the Ethics and Data Monitoring Committee are as follows: Tetsuro Yamane (Department of Surgery, Matsushita Memorial Hospital), Tosiya Sato (Department of Epidemiology and Biostatistics Kyoto University School of Public Health), Yoshiyuki Watanabe (Department of Social Medicine & Cultural Sciences, Research Institute for Neurological Diseases & Geriatrics, Kyoto Prefectural University of Medicine), Hiraku Takebe (Atomic Energy Research Institute, Kinki University). The members of the secretariat are as follows: Ayako Nasu (Osaka Medical Center for Cancer and Cardiovascular Diseases), Takashi Hosaka (Wakunaga Pharmaceutical Co., Ltd.).

## REFERENCES

1. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H, Kawashima T. *Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993; 328: 1797-801.*
2. Kato Y, Nakata K, Omagari K, Furukawa R, Kusumoto Y, Mori I, Tajima H, Tanioka H, Yano M, Nagataki S. *Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Cancer 1994; 74: 2234-8.*
3. Yun TK. *Experimental and epidemiological evidence of the cancer-preventive effects of Panax ginseng C.A. Mayer. Nutr Rev 1996; 54: S71-S81.*
4. Tode T, Kikuchi Y, Kita T, Hirata J, Imaizumi E, Nagata I. *Inhibitory effects by oral administration of ginsenoside Rh2 on the growth of human ovarian cancer cells in nude mice. J Cancer Res Clin Oncol 1993; 120: 24-6.*
5. Nishino H. *Cancer chemoprevention by ginseng in mouse liver and other organs. Proceeding of International Symposium on Cancer Chemoprevention of INSAM (Ginseng) April 20, 2001: 20-1.*
6. Yun TK, Choi SY. *A case-control study of ginseng intake and cancer. Int J Epidemiol 1990; 19: 871-6.*

7. Yun TK, Choi SY. *Preventive effect of ginseng intake against various human cancers: a case-control study on 1987 pairs. Cancer Epidemiol Biomarkers Prev 1995; 4: 401-8.*
8. Oka H, Yamamoto S, Kuroki T, Harihara S, Marumo T, Kim SR, Monna T, Kobayashi K, Tango T. *Prospective study of Chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). Cancer 1995; 76: 743-9.*
9. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Nishikawa M, Seki K, Matsuzawa Y. *Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Ann Intern Med 1998; 129: 94-9.*
10. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. *Randomised trial of effects of interferon-α on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995; 346: 1051-5.*