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# Chemoprevention of Mammary, Cervix and Nervous system Carcinogenesis in Animals using Cultured *Panax ginseng* Drugs and Preliminary Clinical Trials in Patients with Precancerous Lesions of the Esophagus and Endometrium

The anticarcinogenic effects and mechanisms of the biotechnological drugs of *Panax ginseng* C.A. Meyer cultivated in Russia, bioginseng, panaxel and panaxel-5, were studied. Bioginseng was produced from a tissue culture of ginseng root cultured on standard medium, whereas panaxel and panaxel-5 were produced from ginseng tissue root cultures using standard mediums enriched with 2-carboxyethylgermanium sesquioxide and 1-hydroxygermatran-monohydrate respectively. All three ginseng drugs inhibited the development of mammary tumors induced by intramammary injections of N-methyl-N-nitrosourea (MNU) in rats, the development of the brain and spinal cord tumors induced by transplacental administration of N-ethyl-N-nitrosourea (ENU) in rats, and the development of uterine, cervical and vaginal tumors induced by intravaginal applications of 7,12-dimethylbenz(a)anthracene (DMBA) in mice. The ginseng drugs induced the cytotoxic activity of macrophages in mice, enhanced T-lymphocyte rosette formation in guinea pigs exposed to cyclophosphamide, and stimulated the production of thyroid hormones in rats. These mechanisms may contribute to the anticarcinogenic action of the ginseng drugs. The organic germanium compounds present in panaxel and panaxel-5 did not potentiate the anticarcinogenic or immuno-stimulatory effects as much as biogeinseng. Preliminary clinical trials with panaxel and bioginseng were carried out in patients with precancerous lesions of the esophagus and endometrium. Panaxel was found to have a strong therapeutic effect in patients suffering from chronic erosive esophagitis. Bioginseng induced the regression of adenomatous-cystic hyperplasia of the endometrium in some patients. Thus, we conclude that the drugs of ginseng appear to hold considerable promise for future cancer chemoprevention.

Key Words : *Chemical Carcinogenesis; Anticarcinogenic Agents; Ginseng; Precancerous Conditions; Chemoprevention*

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## INTRODUCTION

The use of specific chemicals to prevent the development or retard the progression of carcinogenesis, a technique known as chemoprevention, offers a promising strategy for cancer prevention (1, 2). Cancer chemoprotective potential of naturally occurring phytochemicals in food or medicinal plants continues to be a major area of scientific interest (3, 4). Ginseng has been used for several thousand years in the Orient as a tonic, adaptogenic, anti-aging, prophylactic and restorative remedy. More recently, it has been reported that ginseng has cancer chemopreventive activity (5).

Several studies have shown that some preparations of ginseng inhibit carcinogenesis in animal models. Korean red ginseng extract decreased lung adenoma incidence induced by urethane or aflatoxin B1, diminished the average diameter of lung adenoma and the incidence of diffuse pulmonary infiltration induced by 9,10-dimethyl-1,2-benzanthracene (DMBA), and also decreased the incidence of hepatoma induced by aflatoxin B1 in newborn mice (6). In Yun's anticarcinogenicity test (9 week medium term bioassay model) powders of 6-yr-dried fresh ginseng, 5 and 6 yr-white ginseng, and 4, 5 and 6 yr-red ginseng significantly decreased the incidence of lung adenoma induced by benzo(a)pyrene in newborn N:GP (S) mice (7). In the same mouse model a statistically significant anticarcinogenic effect was observed

in extracts of 6 yr-dried fresh ginseng, 6 yr-white ginseng, and 4, 5 and 6 yr-red ginseng (8). Moreover, a water extract of red ginseng inhibited lung tumor incidence induced by benzo(a)pyrene in newborn N:GP (S) mice (9), and the ethanol-insoluble fraction of the water extract of *Panax ginseng* inhibited lung tumor incidence induced by benzo(a) pyrene in newborn N:GP (S) mice (10). In addition, extract of the roots of *Panax notoginseng* exhibited anti-tumor-promoting activity in a model of the lung carcinogenesis induced by 4-nitroquinoline-N-oxide and glycerol in mice (11). Ginseng extract also inhibited the gastric carcinogenesis induced by N-methyl-N′-nitro-N-nitrosoguanidine in rats (12), and *Panax notoginseng* extract inhibited liver cancer development induced by N-nitrosodiethylamine in rats (13). A saponin, majonoside-R2, isolated from the rhizomes and roots of *Panax vietnamensis* exhibited potent anti-tumor-promoting activity in a two-stage carcinogenic test of mouse hepatic tumor using N-nitrosodiethylamine as an initiator and phenobarbital as a promoter (14). In a two-stage model, red ginseng extracts inhibited DMBA and croton oil-induced skin papilloma in mice (15). The extract of the roots of *Panax notoginseng* exhibited anti-tumor-promoting activity in the two-stage carcinogenesis of mouse skin tumors induced by DMBA as an initiator and a mycotoxin or fumonisin B1 as promoter. This extract exhibited anti-tumor-initiating activity on skin tumors induced by a nitric oxide donor as an initiator and 12-*o*-tetradecanoylphorbol-13-acetate (TPA) as a promoter (16). Topical application of the methanol extract of *Panax ginseng* onto mouse skin 10 min prior to TPA, significantly ameliorated skin papillomagenesis initiated by DMBA (17). The saponin, majonoside-R2, rendered anti-tumor-promoting activity in a two-stage carcinogenic tests of mouse skin using DMBA as an initiator and TPA or fumonisin B1 as a promoter (18). In vitro, the root extract of *Panax ginseng* exerted an inhibitory effect on the transformation of NIH 3T3 cells initiated by 3-methylcholanthrene, methyl methanesulfonate or 1-methyl-3-nitro-1-nitrosoguanidine (19), and the extract of the roots of *Panax notoginseng* (11) and a saponin, majonoside-R2, (18) inhibited the early antigen activation of Epstein-Barr virus induced by TPA in Raji cells.

Several epidemiological studies on ginseng intake and cancer risk have been conducted in the Korea Cancer Center Hospital, Seoul. In a case-control study involving interviewing 905 pairs of cases and controls, ginseng consumers were found to have a lower risk (odds ratio was 0.56) of cancer that ginseng non-consumers (20). Later, Yun et al. extended the number of subjects in a case-controlled study involving 1987 pairs, and showed that increased frequency and duration of ginseng intake decreased the risk of cancer in a dose-dependent manner, and that ginseng consumers had a lower risk for most cancers than non-consumers; these included, cancers of the lip, oral cavity, pharynx, larynx, esophagus, stomach, colon and rectum, liver, pancreas, lung and ovaries (21). The same authors performed a more reliable cohort study in 4634 people over 40 yr old, and showed that ginseng consumers had a lower cancer risk (odds ratio 0.40) than non-consumers, especially for gastric and lung cancers (22).

In the present study, we examined and compared the anti-carcinogenic effects of three biotechnological drugs obtained from *Panax ginseng* C.A. Meyer in mammary, nervous system and cervix animal tumor models induced by chemical carcinogens. We also investigated the immunological and hormonal mechanisms of the ginseng drugs. Preliminary clinical trials of the drugs were also carried out in patients with chronic erosive esophagitis and hyperplasia of the endometrium using the dynamics of precancerous lesions of the esophagus and endometrium as end points.

## MATERIALS AND METHODS

### Chemicals and ginseng drugs

Carcinogens, N-methyl-N-nitrosourea (MNU), N-ethyl-N-nitrosourea (ENU) and 7,12-dimethylbenz(a)anthracene (DMBA), were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ginseng drugs were purchased from Kirishi's Biochemical Factory (Lenigrad region, Russia). It was studied three biotechnological drugs of ginseng root (*Panax ginseng* C.A. Meyer cultivated in Russia), bioginseng, panaxel and panaxel-5 were studied. Bioginseng was produced from a tissue culture of the strain of *Panax ginseng* root cultured in standard medium, which was a modification of the regular medium described by Murashige and Skoog (23). Panaxel and panaxel-5 were produced from tissue cultures of the root cultures of the *Panax ginseng* strain in a standard media enriched with organic germanium compounds, such as 2-carboxyethylgermanium sesquioxide (Ge-132) and 1-hydroxygermatran-monohydrate (23). Bioginseng (24) and panaxel (25) are officinal medicinal drugs. The ginseng drugs used were ethanol extracts of the biomasses of the tissue cultures. The contents of glycosides in bioginseng, panaxel and panaxel-5 were >1.1, >2.2 and >2.5% of the whole alcohol tinctures, respectively. The germanium content of panaxel and panaxel-5 was $10 \times 10^{-3}$ and $2.2 \times 10^{-3}$ mg % to ash respectively. More detailed characteristics of the ginseng drugs used in this study have been described elsewhere (26). In experiments, prior to application, alcohol was removed from the ginseng tinctures with a vacuum evaporator, and water was added to the residue to the volume of the initial tincture. In clinical studies, patients were given ginseng drugs as alcohol tinctures.

### Animals

Outbred albino LIO rats from the Animal Department of

the N.N. Petrov Research Institute of Oncology, St. Petersburg, and outbred albino SHR mice from the Rappolovo Animal Breeding Farm of the Russian Academy of Medical Sciences, Leningrad region, Russia, were used for the study. The characteristics of these rats (27) and mice (28) have been described elsewhere. LIO rats from the Animal Department of the N.N. Petrov Research Institute of Oncology, mice C57Bl and guinea pigs from the Rappolovo Animal Breeding Farm were used in experiments on immunological and hormonal mechanisms. Animals were housed in steel and polypropylene cages, 4-8 animals in each, under a 14 hr/10 hr light/dark regimen at 22±2°C, and received standard laboratory chow and tap water ad libitum.

## Anticarcinogenic experiments

### Mammary carcinogenesis

A model of tumor induction of the mammary gland involving intramammary injections of MNU in rats was used (29). Female LIO 2 month old rats were treated with a single intramammary injection of 1 mg of MNU, dissolved in 0.1 mL saline, per gland into the tissue of all 12 mammary glands. One week after the treatment the rats were randomized and divided into 4 groups. In groups 2, 3 and 4, rats were treated with bioginseng, panaxel or panaxel-5, respectively. The ginseng drugs were given perorally by gavage at 0.5 mL/rat (2.5 mL/kg body weight) for five consecutive days per week for 27 weeks. In group 1 (controls), water was given similarly perorally by gavage at 0.5 mL/rat. Group 5 (intact controls) served as untreated controls. All surviving animals were sacrificed 28 weeks after the beginning of the experiment.

### Transplacental carcinogenesis

A model of induction of tumors of the nervous system and kidney involved the transplacental administration of ENU in rats (30). Pregnant LIO rats, 3-4 months old, were given a single intravenous injection of ENU (75 mg/kg body weight) dissolved in saline, on the 21st day after conception. Their descendants of both sexes were randomized and divided into four groups. In groups 2, 3, and 4 the rats were treated with bioginseng, panaxel or panaxel-5, respectively, perorally by gavage at 0.5 mL/rat (2.5 mL/kg body weight) for five consecutive days, per week, starting at the age of one month for 11 months. In group 1 (control), water was given similarly perorally by gavage at 0.5 mL/rat during their postnatal life. Group 5 (intact controls), descendants of both sexes were obtained from the intact pregnant rats but were not subjected to any treatment during their postnatal life. All surviving offspring were sacrificed 12 months after birth.

### Uterine cervix carcinogenesis

The model of tumor induction of the uterine cervix and vagina in mice involved intravaginal applications of DMBA (29). Female SHR mice, 2 months old, were subjected to intravaginal applications of polyurethane tampons soaked with a solution of 0.1% DMBA in triethylene glycol twice a week for 6 weeks (total dose 300 mkg of DMBA per mouse). After administering the carcinogen, the mice were randomized and divided into eight groups. In the case of groups 1, 2, 3 and 4, the mice were given perorally by gavage, water (control), bioginseng, panaxel or panaxel-5 respectively, at 0.15 mL/mouse (7.5 mL/kg body weight) for five consecutive days weekly over 38 weeks. Groups 5, 6, 7 and 8, were administered daily intravaginal applications of polyurethane tampons soaked with either saline (control), bioginseng, panaxel or panaxel-5 for 16 weeks. The doses of the ginseng drugs were 30 $\mu$L per application. In the group 9 (intact control), the female mice were not exposed to any treatment and served as untreated controls. All surviving mice of the groups 1-4 and 9 were sacrificed 44 weeks after the beginning of experiment. Mice of groups 5-8 were sacrificed 22 weeks after the beginning of the experiment.

### Pathological investigation

All animals sacrificed or found dead before the end of the experiments were autopsied. Animals were killed using ether steams. All tumors and other tissues with macroscopically observed lesions were fixed in 10% neutral formalin, and after routine histological treatment, were embedded in paraffin. Sections (5-to 7-m thick) through the central part of each tumor were stained with haematoxylin and eosin. Neoplasms were classified according to the IARC recommendations (31, 32).

### Investigation of immunological mechanisms

The influence of the ginseng drugs on the cytotoxic and growth-stimulating activities of macrophages was investigated. Male C57Bl mice, 3 months old, were given perorally by gavage, water (control), bioginseng, panaxel or panaxel-5 at 0.15 mL/mouse (7.5 mL/kg body weight) daily for 7 days. After the drug administration period, mice were sacrificed and peritoneal macrophages were collected and pooled from 3-5 animals. These were then incubated in vitro with P3x-63-Ag/8 mouse myeloma cells. The cytotoxic and growth-stimulating phases of macrophage activity were determined as described elsewhere (33), and both of these forms of functional macrophage activity in 5 independent cultures were estimated by [$H^3$] thymidine incorporation into proliferating tumor target cells. Radioactivity was determined using a liquid scintillation counter Delta-300 (Nuclear Chicago, U.S.A.).

The influence of the ginseng drugs on the functional activity of T-system immunity was investigated. In pubertal male guinea pigs with body weights of 250-300 g, T-immunodeficiency was induced with a single subcutaneous injection of cyclophosphamide (Bristol-Myers Squibb, U.S.A.) at a dose of 35 mg/kg of body weight. Thereafter the guinea pigs were randomized and divided into 3 groups. In the control group, the guinea pigs were given water, perorally by gavage at 1 mL daily for 7 days. In the other two groups, the animals were given bioginseng or panaxel, perorally by gavage, at 5 mL/kg of body weight daily for 7 days. In the group of intact controls, the guinea pigs were not subjected to any treatment. The functional activity of T-system immunity was estimated by the method of spontaneous T-cell rosette formation (E-rosettes) of the guinea pig T-lymphocytes with rabbit red blood cells, according to the method described by Stadecker et al. (34).

### Investigation of hormonal mechanisms

The influence of the ginseng drugs on hormonal status was investigated in rats. Male LIO rats, 3 months old, were given, perorally by gavage, water (control), bioginseng, panaxel or panaxel-5 at 0.5 mL/rat (2.5 mL/kg body weight) daily for 7 days. Thereafter, the level of hormones in blood plasma were determined by standard radioimmunoassays. The blood levels of corticotropin and aldosterone were determined using the ACTHK-PR and SB-ALDO-2 kits (ORIS, France) respectively, according to the manufacturer's instructions. The level of corticosterone in blood was determined using the RSL PAT Corticosterone ($H^3$) KIT, RSL, U.S.A. Similarly, the blood level of thyroid-stimulating hormone was determined using the RIA-mat-TSH kit, Mallinkrodt Diagnostica, Germany, and the blood levels of thyroxine and tri-iodothyronine were estimated using the RIO-T4-PG and RIO-T3-PG kits, Minsk, Byelorussia, respectively. At the end of the mammary carcinogenic experiment, the blood from several rats of all groups was collected, and the blood plasma level of estradiol was determined by standard radioimmunoassay using a CIA-TK kit, Sorin, Italy. Radioactivity was determined with $\gamma$- and $\beta$-counters (Beckman, U.S.A.).

### Clinical trials

Preliminary clinical trials of the ginseng drugs were carried out in patients with precancerous lesions of the esophagus and endometrium.

In one clinical study, 64 volunteers suffering from chronic erosive esophagitis were recruited. The patients lived in a region of the Republic of Uzbekistan where the incidence of esophageal cancer and the prevalence of precancerous diseases of the esophagus are elevated. The average age of the patients was $36.5 \pm 3.4$ yr, and there were 41 men and 23 women. The diagnosis of chronic erosive esophagitis was established in the patients based on esophagoscopic examination data. The state of esophageal mucosa of each patient was carefully documented on individual outpatient cards. Inflammatory lesions and multiple erosions of the esophageal mucosa were found in all patients. Erosions were localized to the top third of the esophagus in 4 patients, to the medial third of the esophagus in 15 patients, and simultaneously to the medial and inferior thirds of the esophagus in 45 patients. Patients were given panaxel perorally at 50 drops 3 times every day (approximately 4 mL per day) for 1 month, one course of treatment required approximately 120 mL of the drug. Repeated esophagoscopic examinations were performed 1 month after the start of treatment with panaxel. If the state of esophageal mucosa had not improved as a result of the first course of treatment, patients were given second course of treatment of panaxel under a similar schema. Altogether one, two or three courses of treatment with panaxel were carried out in the patients, after each course of treatment esophagoscopic examination was carried out in each patient, and the endoscopic pattern of the esophageal mucosa was carefully recorded on the individual's outpatient card. The influence of panaxel on the endoscopic appearance of the esophageal mucosa was estimated.

Nineteen control patients suffering from chronic erosive esophagitis (12 men and 7 women; average age $38.2 \pm 5.3$ yr) were also recruited. In all these patients, the inflammatory lesions and multiple erosions of the esophageal mucosa were also found during esophagoscopic examinations. The erosions were localized at the top third of esophagus in 2 patients, at the medial third of the esophagus in 5 patients, and at both the medial and inferior thirds of the esophagus in 12 patients. The control patients did not receive any treatment. Repeated esophagoscopic examinations were performed in all patients after 3 months, and the results of these were carefully recorded on the individual outpatient cards.

In the other clinical study, 11 volunteers suffering from hyperplasia of the endometrium were recruited. The average age of the patients was $42.3 \pm 3.5$ yr, had regular menstrual cycles, and diagnostic curettage of the uterine cavity. Scrapings of the endometrium obtained after routine histological treatment were analyzed under the light microscope. Based on the results of histopathological examinations, adenomatous-cystic hyperplasia of the endometrium was diagnosed in 8 patients. Atypical hyperplasia of the endometrium was found in 3 patients. All patients were given bioginseng perorally at 30 drops 3 times every day (approximately 2.5 mL per day) over a period of 5-6 months (during 6 menstrual cycles). During one course of treatment a patient consumed about 400-450 mL of bioginseng tinctura. After the completion of treatment, repeated diagnostic curettages of the uterus cavity and histolpathological examination of the endometrium were carried out in all patients.

As a control, 9 women with the endometrial hyperplasia

and regular menstrual cycles, 44.1±2.7 yr old, were recruited. Diagnostic curettage of the uterus cavity and histologic examination of the uterine mucosa were performed. Based on the histopathological examinations, diagnosis of adenomatous-cystic hyperplasia of the endometrium was established in 7 patients and atypical hyperplasia of the endometrium in 2 patients. The control patients were not given any treatment, and repeated diagnostic curettages and histolpathological examinations of the uterine mucosa were carried out after 5-6 months (after 6 menstrual cycles)

### Statistics

Statistical analysis was performed using the chi-square test and Student's t-test.

## RESULTS

### Anticarcinogenic experiments with the ginseng drugs

The results on the influence of ginseng drugs on MNU-induced mammary carcinogenesis in rats are shown in Table 1. Intramammary injection of MNU induced tumors of the mammary glands in 77.8% of rats, and the multiplicity of tumors was 1.56 (group 1). The majority of the tumors were adenocarcinomas and in a minority were fibroadenomas. In 22.2% of the animals, mesenchymal tumors of the kidney were induced, which was apparently due to the carcinogenic effect of MNU absorbed after intramammary injections (35). In all the groups, unitary adenomas of the pituitary gland and leukemias developed, which are characteristics of the rat strain used (27) in terms of the spontaneous tumor background. All three ginseng drugs strongly inhibited the mammary carcinogenesis induced by MNU in rats. Compared with the MNU-only control group, bioginseng, panaxel, and panaxel-5 (groups 2, 3 and 4) reduced the incidence of the mammary tumors by 43.3%, 47.0% and 22.2%, respectively, and their respective multiplicities by 62.2%, 60.3% and 33.3%. Similarly, bioginseng, panaxel and panaxel-5 also decreased the incidence of the kidney tumors by 18.8%, 18.4% and 18.5%. In the intact control rats (group 5), the tumors of the mammary gland and of the kidney were not developed.

The results on the influences of the ginseng drugs on the ENU-induced transplacental carcinogenesis in rats are presented in Table 2. Since there were no significant differences between the male and female nervous system and kidney tumor incidences, or between tumor incidences at other sites, the data are presented irrespective of rat sex. ENU mostly induced multiple tumors of the brain, spinal cord, peripheral nervous system and kidneys. Tumors of the peripheral nervous system were mainly localized in the nervi trigemini and rarely in the plexus lumbosacrarilylis, plexus brachialis, radices of the spinal cord or other tissues. We have never found nervous system tumors and kidney tumors developing spontaneously in the rat strain used in this experiment. The incidences of spontaneous tumors of the pituitary gland, mammary gland, ovaries, intestine, and leukemias (group 5) for the rat strain used have been reported before (27). In comparison with the ENU-only control (group 1), the bioginseng (group 2) showed a statistically significant decrease in the total incidence and multiplicity of tumors, by 19.7% and 43.1%, respectively. Moreover, bioginseng also significantly decreased the multiplicity of tumors of the brain and spinal cord by 48.2% and 52.5%, respectively. The anticarcinogenic effects of the germanium-contained drugs of ginseng were expressed slightly less evident. In comparison with the ENU-only control group, panaxel (group 3) and panaxel-5 (group 4) significantly decreased the total multiplicity of tumors by 33.0% and 28.3% respectively, and multiplicity of the brain tumors by 39.9% and 30.6% respectively. In addition, panaxel also reduced the multiplicity of kidney tumors by 55.6%. As a whole, the anticarcinogenic effects of the ginseng drugs on transplacental carcinogenesis can be judged as moderate, as shown primarily by the attitude of tumors of the central nervous system.

The results on the influence of the ginseng drugs on DMBA-induced uterine cervix and vagina carcinogenesis in mice are presented in Table 3. In this carcinogenic model, tumors propagate in the uterine cervix and vagina as a uniform conglomerate. Topical applications of DMBA induced

Table 1. Effects of the drugs of ginseng on the MNU-induced mammary carcinogenesis in female rats

| Group | Treatment | Number of rats | Mammary tumors[a] | | | Incidence of kidney tumors[b] | Incidence of other tumors[c] |
|---|---|---|---|---|---|---|---|
| | | | Tumor incidence | Number of tumors | Average number of tumors/rat (Mean±SD) | | |
| 1 | MNU only, control | 27 | 21 (77.8%) | 42 | 1.56±0.19 | 6 (22.2%) | 3 (11.1%) |
| 2 | MNU+bioginseng | 29 | 10 (34.5%)[d] | 17 | 0.59±0.14[e] | 1 (3.4%)[d] | 1 (3.4%) |
| 3 | MNU+panaxel | 26 | 8 (30.8%)[d] | 16 | 0.62±0.26[e] | 1 (3.8%)[d] | 2 (7.7%) |
| 4 | MNU+panaxel-5 | 27 | 15 (55.6%) | 28 | 1.04±0.15[e] | 1 (3.7%)[d] | 2 (7.4%) |
| 5 | Intact control | 20 | – | – | – | – | 2 (10.0%) |

[a]The most of tumors are adenocarcinomas, a little of tumors are fibroadenomas; [b]Mesenchymal tumors; [c]Unitary adenomas of pituitary gland and leukemias; [d]Statistically significant ($p$<0.05-0.001) compared to the control group given MNU only by the chi-square test; [e]Statistically significant ($p$<0.05-0.001) compared to the control group given MNU only by the Student's t-test.

Table 2. Effects of the drugs of ginseng on the ENU-induced transplacental carcinogenesis in rats

| Group | Treatment | Number of rats | Tumor incidence, number of tumors and average number of tumors/rat (Mean±SD) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | Brain[a] | Spinal cord[a] | Peripheral[b] nerves | Kidney[c] | Others[e] |
| 1 | ENU only, control | 44 | 41 (93.2%)<br>140<br>3.18±0.21 | 36 (81.8%)<br>85<br>1.93±0.17 | 19 (43.2%)<br>27<br>0.61±0.07 | 9 (20.5%)<br>11<br>0.25±0.07 | 15 (34.1%)<br>16<br>0.36±0.07 | 1 (2.3%)<br>1<br>0.02±0.03 |
| 2 | ENU+ bioginseng | 31 | 23 (74.2%)[f]<br>56<br>1.81±0.18[g] | 18 (58.1%)[f]<br>31<br>1.00±0.13[g] | 8 (25.8%)<br>9<br>0.29±0.09[g] | 4 (12.9%)<br>4<br>0.13±0.05 | 7 (22.6%)<br>9<br>0.29±0.09 | 3 (9.7%)<br>3<br>0.10±0.05 |
| 3 | ENU+ panaxel | 38 | 34 (89.5%)<br>81<br>2.13±0.19[g] | 25 (65.8%)<br>44<br>1.16±0.12[g] | 14 (36.8%)<br>16<br>0.42±0.08 | 8 (21.1%)<br>9<br>0.24±0.08 | 6 (15.8%)<br>9<br>0.16±0.04[g] | 5 (13.2%)<br>6<br>0.16±0.08 |
| 4 | ENU+ panaxel-5 | 32 | 28 (87.5%)<br>73<br>2.28±0.17[g] | 21 (65.6%)<br>43<br>1.34±0.13[g] | 12 (37.5%)<br>16<br>0.50±0.09 | 4 (12.5%)<br>4<br>0.12±0.04 | 9 (28.1%)<br>9<br>0.28±0.04 | 1 (3.1%)<br>1<br>0.03±0.04 |
| 5 | Intact control | 30 | 4 (13.3%)<br>5<br>0.17±0.08 | | | | | 4 (13.3%)<br>5<br>0.17±0.08 |

[a]Mainly oligodendrogliomas, mixed oligoastrocytomas, astrocytomas and, rarely, ependymomas, meningiomas and glioblastomas; [b]The majority of the tumors are malignant schwannomas, and in some cases mixed malignant tumors with both sarcomatous and neurogenic component; [c]The most of the tumors are mesenchymal, and in some cases epithelial adenocarcinomas; [e]Unitary adenomas of pituitary gland, leukemias, mammary adenocarcinomas and fibroadenomas, ovarian carcinomas, intestinal adenocarcinomas; [f]Statistically significant ($p$<0.05) compared to the control group given ENU only by the chi-square test; [g]Statistically significant ($p$<0.05-0.001) compared to the control group given ENU only by the Student's t-test.

Table 3. Effects of the drugs of ginseng on DMBA-induced carcinogenesis of the cervix and vagina in female mice

| Group | Treatment | Number of mice | Incidence of the cervix and vagina tumors | | | Incidence of other tumors[c] |
|---|---|---|---|---|---|---|
| | | | Total | Carcinomas[a] | Papillomas[b] | |
| 1 | DMBA only, control | 38 | 26 (68.4%) | 18 (47.4%) | 8 (21.1%) | 6 (15.8%) |
| 2 | DMBA+bioginseng p.o. | 40 | 20 (50.0%) | 15 (37.5%) | 5 (12.5%) | 3 (7.5%) |
| 3 | DMBA+panaxel p.o. | 33 | 19 (57.6%) | 13 (39.4%) | 6 (18.2%) | 3 (9.1%) |
| 4 | DMBA+panaxel-5 p.o. | 35 | 22 (62.9%) | 17 (48.6%) | 5 (14.3%) | 4 (11.4%) |
| 5 | DMBA+saline i.v., control | 38 | 32 (84.2%) | 28 (73.7%) | 4 (10.5%) | 2 (5.3%) |
| 6 | DMBA+bioginseng i.v. | 39 | 22 (56.4%)[d] | 19 (48.7%)[d] | 3 (7.7%) | 2 (5.1%) |
| 7 | DMBA+panaxel i.v. | 21 | 13 (61.9%) | 9 (42.9%)[d] | 4 (19.0%) | 1 (4.8%) |
| 8 | DMBA+panaxel-5 i.v. | 21 | 10 (47.6%)[d] | 8 (38.1%)[d] | 2 (9.5%) | 2 (9.5%) |
| 9 | Intact control | 20 | | – | – | 3 (15.0%) |

p.o.-perorally; i.v.-intravaginally. [a]The majority of the carcinomas are planocellular, and in some cases mixed adeno-planocellular carcinomas and adenocarcinomas; [b]Planocellular papillomas; [c]Unitary adenomas of the lung and adenocarcinomas of the mammary gland; [d]Statistically significant ($p$<0.05-0.001) compared to the control group given DMBA and saline intravaginally by the chi-square test.

tumors of the uterine cervix and vagina in 68.4% of mice, and of 47.4% were carcinomas and 21.1% were papillomas in group 1. Tumors of the uterine cervix and vagina did not develop spontaneously in the mice (group 9). In all the groups, unitary tumors of the lung and mammary gland developed and these were characteristic of the spontaneous tumor background of the mouse strain used (28). All three ginseng drugs, by peroral administration, did not significantly influence the incidence of uterine, cervical or vaginal tumors (groups 2, 3 and 4). In the control group 5, DMBA after co-treatment with tampons soaked with saline induced uterine cervix and vagina tumors much faster than occurred in the DMBA-only control group 1. This is probably linked with tumor promotion by irritation and inflammatory reactions of the cervix and vagina caused by daily intravaginal applications of tampons (36). In comparison with the appropriate control group 5, bioginseng (group 6), panaxel (group 7) and panaxel-5 (group 8) applied topically significantly decreased the total incidence of uterine, cervical and vaginal tumors by 27.8%, 22.3% and 36.6%, respectively, and also the incidence of carcinomas with these localizations by 25.0%, 30.8% and 35.6%, respectively. In conclusion, the ginseng drugs effectively inhibited DMBA-induced uterine, cervical and vaginal carcinogenesis only after intravaginal applications of ginseng drugs, whereas they did not significantly influence the development of these tumors in mice treated perorally.

### Study on the influence of the ginseng drugs on immunological parameters

The results on the influence of the ginseng drugs on the cytotoxic and growth-stimulating activities of macrophages are summarized in Table 4. It was found that all three ginseng drugs induced a strong cytotoxic effect in macrophages on the tumor cells. In comparison with the control, bioginseng, panaxel and panaxel-5 significantly inhibited the proliferative activity of the P3X-63-Ag/8 tumor cells in culture by 58.5%, 65.5% and 58.5%, respectively. None of the ginseng drugs enhanced the growth-stimulating activity of macrophages. Moreover, in this system the growth-stimulating activity of macrophages was suppressed by the drugs. Compared with the control, bioginseng significantly suppressed the growth-stimulating activity of macrophages only by 35.9%, and panaxel and panaxel-5 suppressed this activity only by 15.5% and 12.6%, respectively, not significantly.

The result on the influence of ginseng drugs on the functional activity of T-lymphocytes in guinea pigs are presented in Table 5. Cyclophosphamide produced T-immunodeficiency in animals that showed significant decreases in the numbers of E-rosettes (by 52.6%) and their absolute quantity (by 54.9%) compared with the intact control. Compared with the cyclophosphamide-only group, bioginseng and panaxel significantly raised the relative amount of E-rosettes by 29.9% and 50.2%, respectively, and their absolute quantity by 40.0% and 42.6%, respectively.

Table 4. Influence of the drugs of ginseng on cytotoxic and growth-stimulating activities of macrophages in male mice

| Group | Number of mice | Cytotoxic activity, impulses/hole of plate, minute × $10^2$, (Mean±SD) | Growth-stimulating activity, impulses/hole of plate, minute × $10^2$, (Mean±SD) |
|---|---|---|---|
| Control | 20 | 67.5±9.45 | 51.5±4.12 |
| Bioginseng | 20 | 28.0±3.08[a] | 33.0±3.31[a] |
| Panaxel | 20 | 23.3±2.56[a] | 43.5±4.40 |
| Panaxel-5 | 20 | 28.0±3.36[a] | 45.0± 4.05 |

In the table the data are presented on level of [$H^3$] thymidine incorporation into proliferating tumor target cells of line P3X-63-Ag/8. [a]Statistically significant ($p$<0.001) compared to the control group by the Student's t-test.

### Study on the effect of ginseng drugs on some hormonal parameters

The results of our study on the effect of the ginseng drugs on the hormonal status of male rats are presented in Table 6. All three ginseng drugs stimulated the production of thyroid hormones. Compared with the intact control, bioginseng, panaxel and panaxel-5 significantly raised the blood plasma concentrations of thyroxine by 87.8%, 79.0% and 108.3% and of tri-iodothyronine by 36.1%, 23.6% and 27.8%, respectively. In addition, bioginseng increased the concentration of thyroid-stimulating hormone by 14.5% compared with the intact control group. All three ginseng drugs had no significant influence upon the pituitary-adrenocortical system. The blood plasma levels of corticotropin, aldosterone and corticosterone in all groups were similar.

The results of our study on the influence of the ginseng drugs on estradiol blood plasma levels in female rats exposed to MNU are presented in Table 7. The content of estradiol in the blood of rats increased because of MNU influence. Compared with the intact control, the level of estradiol in blood from rats of the MNU-only group was elevated by

Table 5. Influence of the drugs of ginseng on functional activity of T-lymphocytes in male guinea pigs

| Group | Number of guinea pigs | Relative amount of E-rosettes, % (Mean±SD) | Absolute quantity of E-rosettes, thousands of cells/mg of the thymus tissue, (Mean±SD) |
|---|---|---|---|
| Intact control | 9 | 59.3±0.8 | 266.2±1.9 |
| Cyclophosphamide only | 8 | 28.1±1.7[a] | 120.1±7.9[a] |
| Cyclophosphamide +bioginseng | 8 | 36.5±2.3[b] | 168.1±8.3[b] |
| Cyclophosphamide +panaxel | 8 | 42.2±3.4[b] | 171.3±9.5[b] |

[a]Statistically significant ($p$<0.001) compared to the intact control group by the Student's t-test; [b]Statistically significant ($p$<0.01-0.001) compared to the cyclophosphamide-only group by the Student's t-test.

Table 6. Influence of the drugs of ginseng on the blood plasma level of some hormones in male rats

| Hormone | Blood plasma level of hormone in groups (Mean±SD) | | | |
|---|---|---|---|---|
| | Control (n=15) | Bioginseng (n=12) | Panaxel (n=12) | Panaxel-5 (n=12) |
| Corticotropin, pg/mL | 79.5±10.1 | 68.2±6.8 | 70.1±9.4 | 69.0±7.2 |
| Aldosterone, pg/mL | 81.6±8.3 | 79.1±14.1 | 86.3±12.6 | 73.4±8.5 |
| Corticosterone, ng/mL | 158.8±6.7 | 164.2±10.4 | 171.1±11.5 | 160.3±7.7 |
| Thyroid-stimulating hormone, μU/mL | 0.76±0.03 | 0.87±0.04[a] | 0.75±0.03 | 0.78±0.10 |
| Thyroxine (T4), nmol/L | 43.3±4.5 | 81.3±8.0[a] | 77.5±6.1[a] | 90.2±7.4[a] |
| Tri-iodothyronine (T3), nmol/L | 0.72±0.05 | 0.98±0.09[a] | 0.89±0.0[a] | 0.92±0.07[a] |

n-number of rats. [a]Statistically significant ($p$<0.05-0.001) compared to the intact control group by the Student's t-test.

Table 7. Influence of the drugs of ginseng on the blood plasma level of estradiol in female rats subjected to MNU

| Group | The blood plasma level of estradiol, pmol/L | | | | | |
|---|---|---|---|---|---|---|
| | In all rats | | In rats with mammary tumors | | In rats without mammary tumors | |
| | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| Intact control | 12 | 250±20 | | | | |
| MNU only, control | 23 | 420±67[a] | 17 | 389±49[a] | 6 | 520±230 |
| MNU+ bioginseng | 18 | 370±50[a] | 6 | 508±120[a] | 12 | 303±70 |
| MNU+ panaxel | 21 | 270±34 | 7 | 340±49 | 14 | 230±33[b] |
| MNU+ panaxel-5 | 21 | 310±49 | 10 | 440±91[a] | 11 | 190±28[b] |

n-number of rats. [a]Statistically significant ($p$<0.05-0.01) compared to the intact control group by the Student's t-test; [b]Statistically significant ($p$<0.01-0.001) compared to the MNU-only group, subgroup in rats with mammary tumors, by the Student's t-test.

68.0%. The ginseng drugs reduced this increase in estradiol in blood induced by MNU in rats. In particular, this decline in the blood estradiol level was prominent in animals that received MNU together with the ginseng drugs; these animals developed no tumors of the mammary gland, and should be compared with the subgroup of rats with mammary tumors exposed to MNU only. Bioginseng, panaxel and panaxel-5 reduced the blood estradiol level by 22.1%, 40.9% and 51.2%, respectively, in the MNU-treated rats without mammary tumors.

## Clinical trials of the ginseng drugs on patients with precancerous lesions

Table 8 presents the results of a study on the influence of panaxel on the endoscopic appearance of the esophageal mucosa by esophagoscopy in patients suffering from chronic erosive esophagitis. The results of repeated esophagoscopic examinations revealed that complete regression of erosions and inflammatory lesions of the esophageal mucosa was found in 25 (39.1%) of 64 treated patients 1 month after treatment with panaxel, 2 months after treatment in 10 (25.6%) of 39 treated patients, and 3 months after treatment in 12 (41.1%) of 29 treated patients. In general, treatment with panaxel for 1-3 months induced complete regression of erosions and inflammatory lesions of the esophageal mucosa in 47 (73.4%) of 64 treated patients. In the control group, spontaneous complete regression of erosions and inflammatory lesions of the esophageal mucosa was observed only in 3 (15.8%) of 19 patients after 3 months observation. The difference between the two groups is statistically significant. Therefore, we concluded that panaxel rendered a strong therapeutic effect on the esophageal mucosa of patients with chronic erosive esophagitis.

Table 9 lists the results of our study on the influence of

Table 8. Influence of panaxel on endoscopic appearance of the esophageal mucosa in patients with chronic erosive esophagitis

| Group | Number of patients | Number of patients with | | |
|---|---|---|---|---|
| | | complete regression of erosions and inflammatory lesions | partial regression of erosions and inflammatory lesions | without effect |
| Treatment by panaxel for 1-3 months | 64 | 47 (73.4%)[a] | 13 (20.3%) | 4 (6.3%)[a] |
| Control patients after 3 months of observation | 19 | 3 (15.8%) | 4 (21.1%) | 12 (63.2%) |

[a]Statistically significant ($p$<0.001) compared to the group of control patients which were not given any treatment by the chi-square test.

Table 9. Influence of bioginseng on histologic appearance of the endometrium in patients with the endometrium hyperplasia

| Group | Number of patients | Number of patients with | |
|---|---|---|---|
| | | complete regression of hyperplasia | without effect |
| Treatment by bioginseng for 5-6 months | 11 | 3 (27.3%)[a] | 8 (72.7%) |
| Control patients after 5-6 months of observation | 9 | 0 | 9 (100%) |

[a]Statistically not significant ($p$<0.1) compared to the group of control patients which were not given any treatment by the chi-square test.

bioginseng on the histopathological features of the endometrium in patients with endometrium hyperplasia. The results of histopathological examinations of the endometrium obtained by repeated diagnostic curettages of the uterus demonstrated that after 5-6 months of treatment with bioginseng complete regression of the endometrial hyperplasia was found in 3 (27.3%) of 11 treated patients. These three patients with positive result after panaxel treatment had a diagnosis of adenomatous-cystic hyperplasia prior to the start of the treatment. Bioginseng had no therapeutic effect in patients with atypical hyperplasia of the endometrium. Repeated diagnostic curettages of the uterus and histopathological examinations of the endometrium showed adenomatous-cystic or atypical hyperplasia of the endometrium remained in all 9 control patients 5-6 months after observation. The difference between the two groups was not statistically significant. Thus, long-term treatment with bioginseng seems to regress adenomatous-cystic hyperplasia of the endometrium in some patients.

## Assessment of toxicity

In our study, none of the ginseng drugs was observed to

have a toxic effect on either rats or mice. All animals developed normally and there was no significant difference between the body weights of treated and control animals. In clinical trials patients treated with panaxel or bioginseng did not develop any serious side effects. Two patients treated with panaxel and one patient treated with bioginseng complained of sleep disorders that were probably due to the tonic action of the ginseng drugs.

## DISCUSSION

Our experiments show for the first time that ginseng drugs inhibit the development of tumors of the mammary gland, nervous system, kidney, uterine cervix and vagina as induced by chemical carcinogens in animals. All three ginseng drugs studied showed similar anticarcinogenic effects in the carcinogenic models used. It is important to emphasize that the ginseng drugs inhibited the development of induced tumors of various histogenesis and localizations, such as mammary adenocarcinomas and fibroadenomas, kidney mesenchymal tumors, brain and spinal cord gliomas, uterine cervix and vaginal planocellular carcinomas. When taken together with other earlier reports (6-19), our results suggest that the anticancerogenic action of ginseng is organ non-specific. The results of epidemiological studies (20-22) further confirm the belief that ginseng is capable of inhibiting the development of malignant tumors of many different types. In our experiments, the ginseng drugs were applied in the carcinogenic post-initiation phase. Other authors (6-19) have reported that they render an anticancerogenic action, when applied during the carcinogenic post-initiation phase. Apparently, ginseng is capable of inhibiting the promotion phase of carcinogenesis and early tumor progression.

Ginseng contains a number of biologically active substances, such as triterpenoidal glycosides (ginsenosides), phenolic compounds, sesquiterpenes, alkypyrazine derivatives, neutral or acidic polysaccharides, polyacetylenes and others (4). The identities of its chemical components responsible for its primary anti-carcinogenic effects and their modes of chemopreventive action are not precisely known. It is believed likely that the anticarcinogenic effects of ginseng are due to the cooperative action of biologically active substances and that the mechanisms of tumor development inhibition are complex.

The influence of ginseng on immunity is probably important in terms of its ability to inhibit carcinogenesis. In our experiments, it was shown that the ginseng drugs stimulated some parts of the immune system. Bioginseng, panaxel and panaxel-5 induced the cytotoxic action of macrophages on the tumor cells, but did not enhance the growth-stimulating activity of macrophages. It is also known that such modulation of macrophage activity can suppress tumor development (33). Bioginseng and panaxel also restored the reduced activity of T-lymphocytes caused by cyclophosphamide in guinea pigs. The stimulatory effects of the ginseng drugs on the cytotoxic action of macrophages and T-lymphocytes explain, at least in part, the ability of ginseng to inhibit the promotion of carcinogenesis and an early stage of tumor progression.

Other immunomodulatory mechanisms of ginseng extracts or its active constituents in terms of its promotion of cancer chemoprevention have been reported. Water extract of *Panax ginseng* activated mouse natural killer cells in vitro (10), and red ginseng extract returned natural killer cell activity to a normal level after this was markedly depressed by injections of urethane or benzo(a)pyrene in mice (37). Extracts of *Panax ginseng* enhanced natural killer and the antibody-dependent cell cytotoxicity of peripheral blood mononuclear cells isolated from both normal human individuals and patients with depressed cellular immunity (38). In addition, *Panax ginseng* extracts activated the phagocytosis of peripheral blood polymorphonuclear leucocytes in cows (39) and humans (40), and acidic polysaccharides isolated from the root of *Panax ginseng* showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test (41). Ginsenoside Rg1 from *Panax ginseng* was found to increase the number of spleen plaque-forming cells, the number of antigen-reactive T-cells elicited by sheep red cells in mice, increased the splenocyte natural killer activity, and exerted a direct mitogenic effect on microcultured thymus cells (42).

The influence of ginseng on the hormonal system also could contribute to its ability to inhibit the development of tumors. We demonstrated that bioginseng, panaxel and panaxel-5 administered to male rats for 7 days considerably stimulated the production of the thyroid hormones, thyroxine and tri-iodothyronine. This mechanism may relate to the anticarcinogenic action of ginseng, because it is known that decreased thyroid hormone activity stimulates the development of some tumors in humans and experimental animals (43). In contrast to the above, however, the ginseng drugs did not influence the pituitary-adrenocortical system in the present study, and after 7-days of administration did not influence the blood levels of corticotropin, aldosterone and corticosterone in male rats. Our results are in disagreement with a result that a single administration of ginseng to rats is accompanied by an increase in the basal level of corticotropin and corticosteroids, whereas the basal level of corticotropin and corticosteroids are unchanged after 7-days of administration (44). The stimulation of corticosteroids production should have a great impact on the adaptogenic and the stress-protective actions of ginseng, but the activity of corticotropin and corticosteroids reverts to the basal level after the long-term administration of ginseng.

The present study shows that the blood level of estradiol in female rats treated by MNU was significantly higher than that of intact female rats. The fact that bioginseng, panaxel and panaxel-5 reduced the MNU-induced level of estradiol

in blood level could explain the anticancerogenic action of the ginseng drugs in this carcinogenesis model. It is well known that anti-estrogenic drugs are capable of inhibiting mammary carcinogenesis and that they are applied for prophylaxis and for the treatment of breast cancer (45). It is also known that some plants contain phytoestrogens that blockade estrogen receptors in target-tissues and prohibit the development of estrogen-dependent tumors (46). *Panax quinquefolius* root extract was reported to induce the expression of *pS2*, the estrogen-regulated gene, in MCF-7 breast cancer cells (47). The phytoestrogenic potential of ginseng may contribute to its inhibition of the development of hormone-dependent tumors, such as mammary and endometrium cancers.

Other mechanisms may also contribute to the anticarcinogenic action of ginseng. For example, ginseng has antioxidant properties (48). Ginseng extracts scavenged hydroxyl radicals resulted from iron-mediated lipid peroxidation (49). Ginsenosides Rb1 and Rg1 were found to inhibit the lipid peroxidation of rat liver and brain microsomes, and increase the activities of catalase and GSH peroxidase (50). Panaxadiol ginsenosides extracted from *Panax ginseng* increase the transcription of Cu/Zn superoxide dismutase and catalase genes (51). In smokers treated with red ginseng, plasma antioxidant concentrations increased, while their mean levels of oxidative DNA damage, 8-hydroxydeoxyguanosine, and carbonyl contents decreased (52). Ginseng ginsenosides have anti-inflammatory activity (53), and are capable of inhibiting phosphodiesterase activity, elevating the intracellular level of cyclic AMP (54), and to inducing the differentiation of tumor cells (55). In a recent study, it was shown that the ginsenoside-Rs4, a new ginseng saponin isolated from *Panax ginseng*, elevated the protein levels of p53 and p21WAF1, which are associated with the induction of apoptosis in SK-HEP-1 cells (56).

In our preliminary clinical trials, panaxel was found to have therapeutic effects on the pathological lesions of the esophageal mucosa in patients with chronic erosive esophagitis. These patients lived in a region of the Republic of Uzbekistan with a high incidence of esophageal cancer; chronic esophagitis is considered a precancerous lesion of the esophagus (57). Bioginseng, the other ginseng drug, produced complete regression of adenomatous-cystic hyperplasia of the endometrium in a small number of patients; endometrial hyperplasia is regarded as a precancerous lesion of the endometrium (58). The dynamics of precancerous lesions is one of the surrogate end-point biomarkers in cancer chemopreventive studies (59). The results of our preliminary clinical trials led us to believe that the ginseng drugs should be further studied as agents for the chemoprevention of esophageal cancer in regions of high cancer risk. Further study of the influence of the ginseng drugs on precancerous lesions of the endometrium is recommended.

Organic germanium compounds have been shown to have anticarcinogenic properties in a rat multi-organ carcinogenesis model (60) and in 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats (61). Organic germanium in trace quantities has immuno-enhancing, free radical scavenging and antitumor activities (62). We speculate that the biotechnological drugs of ginseng, containing organic germanium compounds, panaxel and panaxel-5, have greater anticarcinogenic and immuno-stimulatory effects than bioginseng. However, in all the experiments, the anticarcinogenic and immuno-stimulatory actions of the three biotechnological ginseng drugs were effectively equivalent.

In conclusion, the results of our study shows that biotechnological drugs from tissue cultures of *Panax ginseng* C.A. Meyer, namely, bioginseng, panaxel and panaxel-5, have anticarcinogenic activity in different animal chemical carcinogenesis models. The ability of the ginseng drugs to induce the cytotoxic activity of macrophages, to stimulate reactive T-cell immunity, and to improve the production of thyroid hormones may constitute a mechanism of their cancer chemopreventive action. Organic germanium compounds in panaxel and panaxel-5 did not possess more anticarcinogenic or immuno-stimulatory effect than bioginseng. In preliminary clinical trials, panaxel and bioginseng both regressed precancerous lesions in patients with chronic erosive esophagitis and adenomatous-cystic hyperplasia of the endometrium. Therefore, we recommend that ginseng should be recognized as a very promising agent for cancer chemoprevention. Based on the accumulated experimental, epidemiological and clinical results on the cancer chemopreventive activity of ginseng extracts and its active constituents, we suggest that long-term clinical intervention trials design to further our knowledge of the cancer chemopreventive effects of ginseng should be undertaken.

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## REFERENCES

1. Alexandrov VA, Bespalov VG. *Preclinical and clinical study of cancer chemopreventive agents. St. Petersburg: Esculap, 1997; 32 (in Russian).*
2. Kelloff GJ. *Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 2000; 78: 199-334.*
3. Bradlow HL, Telang NT, Sepkovic DW, Osborne MP. *Phytochemicals as modulators of cancer risk. Adv Exp Med Biol 1999; 472: 207-21.*
4. Yun TK. *Update from Asia. Asian studies on cancer chemopreven-*

*tion. Bradlow HL, Fishman J, Osborne MP, editors, Cancer prevention. Novel nutrient and pharmaceutical developments. Ann NY Acad Sci 1999; 889: 157-92.*

5. Yun TK. *Experimental and epidemiological evidence of the cancer-preventive effects of Panax ginseng C.A. Meyer. Nutr Rev 1996; 54: S71-81.*
6. Yun TK, Yun YS, Han IW. *Anticarcinogenic effect of long-term oral administration of red ginseng on newborn mice exposed to various chemical carcinogens. Cancer Detect Prev 1983; 6: 515-25.*
7. Yun TK, Lee YS. *Anticarcinogenic effect of ginseng powders depending on the types and ages using Yun's anticarcinogenicity test (I). Korean J Ginseng Sci 1994; 18: 89-94.*
8. Yun TK, Lee YS. *Anticarcinogenic effect of ginseng extracts depending on the types and ages using Yun's anticarcinogenicity test (II). Korean J Ginseng Sci 1994; 18: 160-4.*
9. Yun TK, Kim SH, Lee YS. *Trial of a new medium-term model using benzo(a)pyrene induced lung tumor in newborn mice. Anticancer Res 1995; 15: 839-45.*
10. Yun YS, Lee YS, Jo SK, Jung IS. *Inhibition of autochthonous tumor by ethanol insoluble fraction from Panax ginseng as an immunomodulator. Planta Med 1993; 59: 521-4.*
11. Konoshima T, Takasaki M, Tokuda H. *Anti-tumor-promoting activities of the roots of Panax notoginseng (1). Nature Med 1996; 50: 158-62.*
12. Kim JP, Park JG, Lee MD, Han MD, Park ST, Lee BH, Jung SE. *Co-carcinogenic effects of several Korean foods on gastric cancer induced by N-methyl-N'-nitro-N-nitrosoguanidine in rats. Jpn J Surg 1985; 15: 427-37.*
13. Wu XG, Zhu DH. *Influence of ginseng upon the development of liver cancer induced by diethylnitrosamine in rats. J Tongji Med Univ 1990; 10: 141-5, 133.*
14. Konoshima T, Takasaki M, Ichiishi E, Murakami T, Tokuda H, Nishino H, Duc NM, Kasai R, Yamasaki K. *Cancer chemopreventive activity of majonoside-R2 from Vietnamese ginseng, Panax vietnamensis. Cancer Lett 1999; 147: 11-6.*
15. Xiaoguang C, Hongyan L, Xiaohong L, Zhaodi F, Yan L, Lihua T, Rui H. *Cancer chemopreventive and therapeutic activities of red ginseng. J Ethnopharmacol 1998; 60: 71-8.*
16. Konoshima T, Takasaki M, Tokuda H. *Anti-carcinogenic activity of the roots of Panax notoginseng. II. Biol Pharm Bull 1999; 22: 1150-2.*
17. Keum YS, Park KK, Lee JM, Chun KS, Park JH, Lee SK, Kwon H, Surh YJ. *Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Lett 2000; 150: 41-8.*
18. Konoshima T, Takasaki M, Tokuda H, Nishino H, Duc NM, Kasai R, Yamasaki K. *Anti-tumor-promoting activity of majonoside-R2 from Vietnamese ginseng, Panax vietnamensis Ha et Grushv. (I). Biol Pharm Bull 1998; 21: 834-8.*
19. Rhee YH, Ahn JH, Choe J, Kang KW, Joe C. *Inhibition of mutagenesis and transformation by root extracts of Panax ginseng in vitro. Planta Med 1991; 57: 125-8.*
20. Yun TK, Choi SY. *A case-control study of ginseng intake and cancer. Int J Epidemiol 1990; 19: 871-6.*
21. 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.*
22. Yun TK, Choi SY. *Non-organ specific cancer prevention of ginseng: a prospective study in Korea. Int J Epidemiol 1998; 27: 359-64.*
23. Slepyan L, Jouravleva D, Galynkin V, Yakovlev G. *Biotechnology of ginseng tissue culure cells as a model for development of new ginseng preparation. In: International Ginseng Conference, Ginseng: Its Sciences and Its Markets. Hong Kong, China, 1999; 52.*
24. Bioginseng. *Officinal Article 42-1890.89 on 21.04.89 (in Russian).*
25. Panaxel. *Officinal Article 42-3044-98 on 13.10.1998 (in Russian).*
26. Slepyan L, Antan I, Phedorova V, Kudrina L, Vasil'eva N. *Strains of ginseng and other araliaceae and their usage. In: Bailey WG, Whitehead C, Proctor JTA, Kyle JT, editors. Proceedings of the International Ginseng Conference-Vancouver 1994. Canada, Burnaby, British Columbia: Simon Fraser University, 1995; 116-9.*
27. Anisimov VN, Pliss GB, Iogannsen MG, Popovich IG, Romanov KP, Monakchov AS, Averyanova TK. *Spontaneous tumors in outbred LIO rats. J Exp Clin Cancer Res 1989; 8: 254-62.*
28. Anisimov VN, Loktionov AS, Khavinson VK, Morozov VG. *Effect of low-molecular-weight factors of thymus and pineal gland on life span and spontaneous tumor development in female mice of different age. Mech Ageing Dev 1989; 49: 245-57.*
29. Bespalov VG, Petrov AS, Troyan DN, Alexandrov VA. *Anticarcinogenic effect of theophylline on the development of induced tumors in animals. Experim Oncol 1993; 15: 23-7(in Russian).*
30. Alexandrov VA, Bespalov VG, Boone CW, Kelloff GJ, Malone WF. *Study of postnatal effects of chemopreventive agents on offspring of ethylnitrosourea-induced transplacental carcinogenesis in rats. I. Influence of retinol acetate, α-tocopherol acetate, thiamine chloride, sodium selenite, and α-difluoromethylornithine. Cancer Lett 1991; 60: 177-84.*
31. Turusov VS, Mohr U, editors. *Pathology of tumours in laboratory animals, Second edition, Volume 1, Tumours of the rat. IARC Sci Publ No 99. Lyon, France: International Agency for Research on Cancer, 1990; 748.*
32. Turusov VS, Mohr U, editors. *Pathology of tumours in laboratory animals, Second edition, Volume 2, Tumours of the mouse. IARC Sci Publ No 111. Lyon, France: International Agency for Research on Cancer, 1994; 776.*
33. Okulov VB, Voytenkov BO, Ushmorov AG, Polischuk ND, Gromov SA. *Growth-stimulating phase of macrophage response to activation: the phenomenon and its implications for tumour growth and immunotherapy. J Cancer Res Clin Oncol 1992; 118: 537-41.*
34. Stadecker MJ, Bishop J, Wortis HH. *Rosette formation by guinea pigs thymocytes and thymus derived lymphocytes with rabbit red blood cells. J Immunol 1974; 111: 1834-56.*
35. Druckrey H, Preussmann R, Ivankovic S, Schmahl D. *Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten. Z Krebsforsch 1967; 69: 103-201(in German).*
36. Cerutti PA. *Response modification creates promotability in multistage carcinogenesis. Carcinogenesis 1988; 9: 519-26.*
37. Yun YS, Moon HS, Oh YR, Jo SK, Kim YJ, Yun TK. *Effect of red ginseng on natural killer cell activity in mice with lung adenoma induced by urethan and benzo(a)pyrene. Cancer Detect Prev Suppl*

*1987; 1: 301-9.*

38. See DM, Broumand N, Sahl L, Tilles JG. *In vitro effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. Immunopharmacology 1997; 35: 229-35.*
39. Hu S, Concha C, Cooray R, Holmberg O. *Ginseng-enhanced oxidative and phagocytic activities of polymorphonuclear leucocytes from bovine peripheral blood and stripping milk. Vet Res 1995; 26: 155-61.*
40. Scaglione F, Ferrara F, Dugnani S, Falchi M, Santoro G, Fraschini F. *Immunomodulatory effects of two extracts of Panax ginseng C.A. Meyer. Drugs Exp Clin Res 1990; 16: 537-42.*
41. Tomoda M, Hirabayashi K, Shimizu N, Gonda R, Ohara N, Takada K. *Characterization of two novel polysaccharides having immunological activities from the root of Panax ginseng. Biol Pharm Bull 1993; 16: 1087-90.*
42. Kenarova B, Neychev H, Hadjiivanova C, Petkov VD. *Immunomodulating activity of ginsenoside Rg1 from Panax ginseng. Jpn J Pharmacol 1990; 54: 447-54.*
43. Rao GN. *Influence of diet on tumors of hormonal tissues. Prog Clin Biol Res 1996; 394: 41-56.*
44. Filaretov AA, Bogdanova TS, Podvigina TT, Bodganov AI. *Role of pituitary-adrenocortical system in body adaptation abilities. Exp Clin Endocrinol 1988; 92: 129-36.*
45. Chlebowski RT, Collyar DE, Somerfield MR, Pfister DG. *American Society of Clinical Oncology technology assessment on breast cancer risk reduction strategies: tamoxifen and raloxifene. J Clin Oncol 1999; 17: 1939-55.*
46. Kurzer MS, Xu X. *Dietary phytoestrogens. Annu Rev Nutr 1997; 17: 353-81.*
47. Duda RB, Zhong Y, Navas V, Li MZ, Toy BR, Alavarez JG. *American ginseng and breast cancer therapeutic agents synergistically inhibit MCF-7 breast cancer cell growth. J Surg Oncol 1999; 72: 230-9.*
48. Gillis CN. *Panax ginseng pharmacology: a nitric oxide link? Biochem Pharmacol 1997; 54: 1-8.*
49. Zhang D, Yasuda T, Yu Y, Zheng P, Kawabata T, Ma Y, Okada S. *Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. Free Radic Biol Med 1996; 20: 145-50.*
50. Deng HL, Zhang JT. *Anti-lipid peroxilative effect of ginsenoside Rb1 and Rg1. Chin Med J Engl 1991; 104: 395-8.*
51. Chang MS, Lee SG, Rho HM. *Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from Panax ginseng. Phytother Res 1999; 13: 641-4.*
52. Lee BM, Lee SK, Kim HS. *Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, beta-carotene and red ginseng). Cancer Lett 1998; 132: 219-27.*
53. Matsuda H, Samukawa K, Kubo M. *Anti-inflammatory activity of ginsenoside Ro. Planta Med 1990; 56: 19-23.*
54. Stancheva SL, Alova LG. *Ginsenoside Rg1 inhibits the brain cAMP phosphodiesterase activity in young and aged rats. Gen Pharmacol 1993; 24: 1459-62.*
55. Lee YN, Lee HY, Chung HY, Kim SI, Lee SK, Park BC, Kim KW. *In vitro induction of differentiation by ginsenoides in F9 teratocarcinoma cells. Eur J Cancer 1996; 32A: 1420-8.*
56. Kim SE, Lee YH, Park JH, Lee SK. *Ginsenoside-Rs4, a new type of ginseng saponin concurrently induces apoptosis and selectively elevates protein levels of p53 and p21WAF1 in human hepatoma SK-HEP-1 cells. Eur J Cancer 1999; 35: 507-11.*
57. Kavin H, Yaremko L, Valaitis J, Chowdhury L. *Chronic esophagitis evolving to verrucous squamous cell carcinoma: possible role of exogenous chemical carcinogens. Gastroenterology 1996; 110: 904-14.*
58. Burke TW, Tortolero-Luna G, Malpica A, Baker VV, Whittaker L, Johnson E, Follen-Mitchell M. *Endometrial hyperplasia and endometrial cancer. Obstet Gynecol Clin North Am 1996; 23: 411-56.*
59. Kelloff GJ, Sigman CC, Hawk ET, Johnson KM, Crowell JA, Guyton KZ. *Surrogate end-point biomarkers in chemopreventive drug development. In: Miller AB, Bartsch H, Boffetta P, Dragsted L, Vainio H, editors, Biomarkers in cancer chemoprevention. IARC Sci Publ No 154. Lyon: International Agency for Research on Cancer, 2001; 154: 13-26.*
60. Jang JJ, Cho KJ, Lee YS, Bae JH. *Modifying responses of allyl sulfide, indole-3-carbinol and germanium in a rat multi-organ carcinogenesis model. Carcinogenesis 1991; 2: 691-5.*
61. Jao SW, Lee W, Ho YS. *Effect of germanium on 1,2-dimethylhydrazine-induced intestinal cancer in rats. Dis Colon Rectum 1990; 33: 99-104.*
62. Goodman S. *Therapeutic effects of organic germanium. Med Hypotheses 1988; 26: 207-15.*