Микроэлементы в медицине 3(3): 41–44

METAL IONS

ANTICARCINOGENIC AND ANTITUMOR ACTIVITIES OF GERMANIUM-SELECTIVE DRUGS OF GINSENG

V.G. Bespalov[1], V.A. Alexandrov[1], L.V. Mironova[1], A.Yu. Limarenko[1], L.I. Slepyan[2], V.V. Davydov[2]

[1] N.N. Petrov Research Institute of Oncology of the Ministry of Health of the Russian Federation.
[2] State Chemical-Pharmaceutical Academy, St. Petersburg, Russia.

Introduction

Germanium in micro-trace quantities have been reported to have immuno-stimulatory, free radical scavenging and antitumor actions. We examined the anticarcinogenic and antitumor effects of gernanium-selective drugs of ginseng. The drugs, panaxel and panaxel-5, were produced from biomasses of tissue cultures of the strain of *Panax ginseng* root cultured on the standard mediums enriched with organic compounds of germanium, accordingly 2-carboxyethylgermanium sesquioxide (Ge-132) and 1-hydroxygermatran-monohydrat. The germanium content of panaxel and panaxel-5 was 10×10^{-3} and 2.2×10^{-3} mg % to ash, respectively. Anticarcinogenic effects of the drugs were studied in models of murine tumors induced by chemical carcinogens. Antitumor effects of the drugs were studied in mice transplanted with sarcoma-180 or Lewis lung carcinoma. Panaxel and panaxel-5 were given to animals perorally, as ethanol extracts, after carcinogen exposure or tumor transplantation. The both drugs inhibited the development of the mammary adenocarcinomas and fibroadenomas induced by intramammary injections of N-methyl-N-nitrosourea (MNU) in rats, the development of the brain and spinal cord gliomas induced by transplacental administration of N-ethyl-N-nitrosourea (ENU) in rats, and the development of the uterine cervix and vagina adenocarcinomas induced by intravaginal applications of 7,12-dimethylbenz(a)anthracene (DMBA) in mice. Panaxel and panaxel-5 inhibited the growth of sarcoma-180 or Lewis lung carcinoma in mice. The drugs also decreased a number of lung metastases of Lewis lung carcinoma transplanted into pad of mice. Thus, panaxel and panaxel-5 have anticarcinogenic and antitumor actions. The organogermanium compounds potentiate the efficacy of biologically active substances of ginseng. The germanium-selective drugs of ginseng appear to hold considerable promise for cancer chemoprevention and chemotherapy. Germanium-containing remedies became popular in the 1970s in Japan and later in other countries, as drugs for certain diseases, for example cancer and AIDS (Tao, Bolger, 1997). Germanium is not an essential element. Organic germanium compounds in trace quantities have immuno-enhancing, free radical scavenging and antitumor activities (Goodman, 1988). Ginseng has been used as a tonic, adaptogenic, prophylactic and restorative remedy. More recently, it has been reported that ginseng has cancer chemopreventive (Yun et al., 2001) and therapeutic actions (Mantle et al., 2000). The purpose of our work was study of anticarcinogenic, antitumor and antimetastatic activities of biotechnological germanium-selective drugs of ginseng and comparison of them with a drug of ginseng without germanium.

Materials and Methods

Carcinogens, MNU, ENU and DMBA, were obtained from Sigma Chemical Co. (USA). Drugs of ginseng were purchased from the Kirishi's Biochemical Factory (Lenigrad region, Russia). It was studied three biotechnological drugs of ginseng root (*Panax ginseng* C.A. Meyer), bioginseng, panaxel and panaxel-5. Bioginseng is produced from a biomass of tissue culture of the strain of *Panax ginseng* root cultured on standard medium. Panaxel and panaxel-5 are produced from a biomasses of tissue cultures of the strain of *Panax ginseng* root cultured on the standard mediums enriched with organic compounds of germanium, accordingly 2-carboxyethylgermanium sesquioxide (Ge-132) and 1-hydroxygermatran-monohydrat. The drugs of ginseng have been used as ethanol extracts from biomasses of the tissue cultures. The contents of germanium in panaxel and panaxel-5 are corrrespondingly 10×10^{-3} and 2.2×10^{-3} mg% to ashes. Directly ahead of the application, alcohol was deleted from the ginseng tinctures with the vacuum evaporator, and water was added to the remnant until an equal volume of initial tincture. LIO rats from the Animal Department of the N.N. Petrov Research Institute of Oncology, St. Petersburg, and SHR and C_{57}Bl mice from the Rappolovo Animal Breeding Farm of the Russian Academy of Medical Sciences, Leningrad region, Russia, were used in experiments. Animals were kept in steel and polypropylene cages, 4–8 in each, under a 14h/10h light/dark regimen at 22 ± 2°C. They received standard laboratory chow and tap water.

In the mammary carcinogenesis experiment, female rats at the age of 2 months were administered single

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

Treatment	Number of rats	Mammary tumor incidence	Average number of mammary tumors/rat (Mean ± SD)	Incidence of kidney tumors
MNU only, control	27	21 (77.8%)	1.56 ± 0.19	6 (22.2%)
MNU + bioginseng	29	10 (34.5%)[a]	0.59 ± 0.14[b]	1 (3.4%) [a]
MNU + panaxel	26	8 (30.8%)[a]	1.62 ± 0.26[b]	2 (7.7%) [a]
MNU + panaxel-5	27	15 (55.6%)	1.04 ± 0.15[b]	2 (7.4%) [a]

In this and other tables:
[a] Statistically significant ($p<0.005–0.001$) compared to the control group by the chi-square test.
[b] Statistically significant ($p<0.005–0.001$) compared to the control group by the Student-test.

intramammary injections of MNU into a tissue of all 12 mammary glands at 1 mg of MNU, dissolved in 0.1 ml saline, per gland. One week after the carcinogen exposure the rats were randomized and divided into 4 groups. In control group the rats were given water perorally by gavage at 0.5 ml/rat. In other groups the rats were treated with bioginseng, panaxel or panaxel-5, respectively. The drugs of ginseng were given perorally by gavage at 0.5 ml/rat (2.5 ml/kg body weight) for five consecutive days weekly during 27 weeks. All surviving animals were sacrificed 28 weeks after the beginning of experiment.

In the transplacental carcinogenesis experiment, pregnant rats, 3–4 months old, were given a single intravenous injection of ENU dissolved in saline, 75 mg/kg body weight, on the 21st day after conception. Their descendants of both sexes were randomized and divided into 4 groups. In control group the rats were given water perorally by gavage at 0.5 ml/rat during their postnatal life. In other groups 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 weekly starting at the age of one month during 11 months. All surviving rat offsprings were sacrificed 12 months after birth.

In the uterine cervix carcinogenesis experiment, female SHR mice, 2 months old, were subjected to intravaginal applications of polyuretane tampons soaked with 0.1 % triethylene glycol solution of DMBA two times a week for 6 weeks (total dose was 300 mkg of DMBA per mouse). After the end of carcinogen applications the mice were randomized and divided into 4 groups. In the groups the mice were administered daily with intravaginal applications of polyuretane tampons soaked correspondingly with saline (control), bioginseng, panaxel or panaxel-5 during 16 weeks. The doses of the drugs of ginseng were 30 ml per one application. All surviving mice were sacrificed 22 weeks after the beginning of experiment.

All animals sacrificed or found dead before the end of the experiments were autopsied. Animals were killed without pain by ether steams. All tumors and other tissues with macroscopically revealed lesions were fixed in 10% neutral formalin, and after routine histological treatment, were embedded in paraffin. Sections through the central part of each tumor were stained with haematoxylin and eosin and analyzed by microscopy.

The antitumor activity of the drugs of ginseng was studied on models of transplanted tumors, sarcoma-180 transplanted to female mice SHR, and Lewis lung carcinoma transplanted to female mice C_{57}BL. In the mice 10^6 of tumor cells were inoculated into foot of a back pad. Dimensions of a tumor were determined from the date of its appearance to the terminal of experiments. Mass of a tumor was calculated under the specially developed formula proceeding from thickness of foot. Bioginseng, panaxel and panaxel-5 were given perorally by gavage at 0.25 ml/mouse (12.5 ml/kg body weight) daily from 3th until to 14th day of experiment. For study of antimetastatic activity of the drugs, Lewis lung carcinoma in quantity 2×10^5 of tumor cells were inoculated into foot of a back pad in mice C_{57}BL. Two weeks after tumor inoculation a pad with tumor was amputated, and since the next day bioginseng, panaxel and panaxel-5 were given in the same doses during 14 days. After the terminal of the drug administration, mice were sacrificed, number of the lung metastases was counted including large, more than 2 mm in a diameter. In control groups, mice were given water perorally by gavage.

For statistical analysis chi-square test and Student's t-test were used.

Results

The results of study of anticarcinogenic effects of the ginseng drugs are shown in Tables 1–3.

MNU induced tumors of the mammary glands in 77.8 % of rats, multiplicity of the tumors was 1.56. On histological type, the majority of tumors were adenocarcinomas; in small number of cases they were fibroadenomas. At 22.2 % of animals the mesenchymal tumors of the kidney were induced that apparently is a consequence of action of MNU absorbed after intramammary injections and propely to its carcinogenic effect. The all three drugs of ginseng strongly inhibited the mammary carcinogenesis induced by MNU in rats. In comparison with the MNU-only control group, bioginseng, panaxel, and panaxel-5 reduced the incidence of the mammary tumors by 43.3 %, 47.0 % and 22.2 %, respectively, and their multiplicity by 62.2 %, 60.3 % and 33.3 %, respectively. Bioginseng, panaxel and panaxel-5 also decreased the incidence of the kidney tumors by 18.8 %, 18.4 % and 18.5 %, respectively (Tab. 1).

TABLE 2. EFFECTS OF THE DRUGS OF GINSENG ON THE ENU-INDUCED TRANSPLACENTAL CARCINOGENESIS IN RATS.

Treatment	Number of rats	Tumor incidence and number of tumors/rat (Mean ± SD)				
		Total	Brain	Spinal cord	Peripheral nerves	Kidney
ENU only, control	44	41 (93.2%) 3.18 ± 0.21	36 (81.8%) 1.93 ± 0.17	19 (43.2%) 0.61 ± 0.07	9 (20.5%) 0.25 ± 0.07	15 (34.1%) 0.36 ± 0.07
ENU + bioginseng	31	23 (74.2%)[a] 1.81 ± 0.18b	18 (58.1%)[a] 1.00 ± 0.13[b]	8 (25.8%) 0.29 ± 0.09[b]	4 (12.9%) 0.13 ± 0.05	7 (22.6%) 0.29 ± 0.09
ENU + panaxel	38	34 (89.5%) 2.13 ± 0.19[b]	25 (65.8%) 1.16 ± 0.12[b]	14 (36.8%) 0.42 ± 0.08	8 (21.1%) 0.24 ± 0.08	6 (15.8%) 0.16 ± 0.04[b]
ENU + panaxel-5	32	28 (87.5%) 2.28 ± 0.17[b]	21 (65.6%) 1.34 ± 0.13[b]	12 (37.5%) 0.50 ± 0.09	4 (12.5%) 0.12 ± 0.04	9 (28.1%) 0.28 ± 0.04

TABLE 3. EFFECTS OF THE DRUGS OF GINSENG ON THE DMBA-INDUCED CARCINOGENESIS OF THE CERVIX AND VAGINA IN MICE.

Treatment	Number of mice	Incidence of the cervix and vagina tumors		
DMBA only, control	38	32 (84,2%)	28 (73.7%)	4 (10.5%)
DMBA + bioginseng	39	22 (56.4%)[a]	19 (48.7%)[a]	3 (7.7%)
DMBA + panaxel	21	13 (61.9%)	9 (42.9%)[a]	4 (19.0%)
DMBA + panaxel-5	21	10 (47.6%)[a]	8 (38.1%)[a]	2 (9.5%)

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 lumbosacralis, plexus brachialis, radices of the spinal cord or other tisuues. In comparison with the ENU-only control, bioginseng statistically significantly decreased total incidence and multiplicity of the tumors by 19.7 % and 43.1 %, respectively, this drug also significantly decreased multiplicity of tumors of the brain and spinal cord by 48.2 % and 52.5 % respectively. The anticarcinogenic effects of germanium-contained drugs of ginseng were expressed slightly less evident. In comparison with the ENU-only control group, panaxel and panaxel-5 statistically significantly decreased total multiplicity of tumors by 33.0 % and 28.3 %, respectively, and multiplicity of the brain tumors by 39.9 % and 30.6 %, respectively; panaxel reduced also multiplicity of the kidney tumors by 55.6 % (Tab. 2).

DMBA induced tumors of the uterine cervix and vagina in 84.2 % of mice; from them there are carcinomas in 73.7 % of mice and papillomas in 10.5 % of mice. In comparison with the DMBA-only control group, bioginseng, panaxel and panaxel-5 at topical applications statistically significantly decreased total incidence of the uterine cervix and vagina tumors by 27.8 %, 22.3 % and 36.6 %, respectively, and also the incidenece of carcinomas of this localization by 25.0 %, 30.8 % and 35.6 %, respectively (Tab. 3).

The results of study of antitumor and antimetatstatic effects of the ginseng drugs are shown in Tables 4–5.

Bioginseng exerted not significant tendency to a

TABLE 4. EFFECTS OF THE DRUGS OF GINSENG ON THE GROWTH OF TRANSPLANTED TUMORS IN MICE.

Treatment	Number of mice	Mass of tumor (M ± m), mg (% of tumor growth inhibition)					
		Day after tumor inoculation					
		7th	9th	11th	12th	13th	14th
Transplanted tumor		Sarcoma-180					
Control	9	59 ± 13	132 ± 7	287 ± 66	343 ± 66		572 ± 68
Bioginseng	10	72 ± 16	111 ± 17	164 ± 23	197 ± 43		261 ± 33[b] (54%)
Panaxel	10	17 ± 6[b] (80%)	68 ± 11[b] (48%)	93 ± 17[b] (68%)	151 ± 30[b] (56%)		189 ± 23[b] (67%)
Panaxel-5	10	55 ± 13	90 ± 14	103 ± 21[b] (64%)	169 ± 37[b] (51%)		202 ± 44[b] (65%)
Transplanted tumor		Lewis lung carcinoma					
Control	10	54 ± 5	83 ± 8			153 ± 15	
Bioginseng	9	47 ± 11	56 ± 19			81 ± 29	
Panaxel	9	20 ± 5[b] (63%)	33 ± 8[b] (60%)			42 ± 10[b] (73%)	
Panaxel-5	9	36 ± 4[b] (33%)	40 ± 8[b] (52%)			50 ± 7[b] (67%)	

Table 5. Effects of the drugs of ginseng on metastasizing of Lewis lung carcinoma in mice.

Treatment	Number of mice	Number of lung metastases/mouse (M ± m)	
		Total	> 2 mm in diameter
Control	9	36.4 ± 7.2	31.6 ± 6.1
Bioginseng	10	26.4 ± 3.9	22.7 ± 3.1
Panaxel	10	20.2 ± 4.0b	17.2 ± 3.2b
Panaxel-5	10	19.6 ± 3.3b	18.0 ± 3.3b

growth inhibition of both strains of transplanted tumors. Only for 14th day after inoculation of sarcoma-180, mass of tumor was significantly less by 54 % in comparison with the control. Panaxel and panaxel-5 practically in all terms of dimensions of tumors significantly inhibited the growth sarcoma-180 and Lewis lung carcinoma by 33–80% compared with the control (Tab. 4).

Bioginseng exerted not significant tendency to depression of metastasizing Lewis lung carcinoma. Panaxel and panaxel-5 significantly decreased number of the lung metastases, as total and large, by 43–46% in comparison with the control (Tab. 5).

Discussion

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 that caused anticarcinogenic and antitumor effects (Yun et al., 2001). Organic germanium compounds was shown to have anticarcinogenic properties in a rat multi-organ carcinogenesis model (Jang et al., 1991) and in 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats (Jao et al., 1994). We speculate that the drugs of ginseng containing organic germanium compounds, panaxel and panaxel-5, have greater anticarcinogenic effects than bioginseng. However, in all experiments, the anticarcinogenic actions of the three compared biotechnological drugs of ginseng were effectively equivalent. Ge-132 have passed phase II clinical studies and have shown cytostatic activity against certain human carcinomas and lymphomas (Kopf-Maier, 1994). Germanium compounds stimulate antitumor immune reactions and inhibit tumor growth as biologic response modifiers (Fukazawa et al., 1994). In our experiments, the drugs of ginseng containing organic germanium compounds, panaxel and panaxel-5, had greater antitumor and antimetastatic effects than bioginseng.

Conclusion

The results of our study show that biotechnological germanium-selective drugs from tissue culture of *Panax ginseng* C.A. Meyer, panaxel and panaxel-5, have anticarcinogenic activity in different models of chemical carcinogenesis in animals. Organic germanium compounds in panaxel and panaxel-5 did not possess more anticarcinogenic effect than bioginseng, drug without germanium. On the other hand, panaxel and panaxel-5 have antitumor and antimetastatic activities in models of transplanted tumors in mice. Organic germanium compounds potentiate the antitumor and antimetastatic effects. The germanium-selective drugs of ginseng are perspective for cancer chemoprevention and chemotherapy.

References

Fukazawa H., Ohashi Y., Sekiyama S., Hoshi H., Abe M., Takahashi M., Sato T. 1994. Multidisciplinary treatment of head and neck cancer using BCG, OK-432, and GE-132 as biologic response modifiers // Head Neck. Vol.16. P.30–38.

Goodman S. 1988. Therapeutic effects of organic germanium // Med. Hypotheses. Vol.26. P.207–215.

Jang J.J., Cho K.J., Lee Y.S., Bae J.H. 1991. Modifying responses of allyl sulfide, indole-3-carbinol and germanium in a rat multi-organ carcinogenesis model // Carcinogenesis. Vol.2. P.691–695.

Jao S.W., Lee W., Ho Y.S. 1990. Effect of germanium on 1,2-dimethylhydrazine-induced intestinal cancer in rats // Dis. Colon Rectum. Vol.33. P.99–104.

Kopf-Maier P. 1994. Complexes of metals other than platinum as antitumour agents // Eur. J. Clin. Pharmacol. Vol.47. P.1–16.

Mantle D., Lennard T.W., Pickering A.T. 2000. Therapeutic applications of medicinal plants in the treatment of breast cancer: a review of their pharmacology, efficacy and tolerability // Adverse Drug React. Toxicol. Rev. Vol.19. P.223–240.

Tao S.H., Bolger P.M. 1997. Hazard assessment of germanium supplements // Regul. Toxicol. Pharmacol. Vol.25. P.211–219.

Yun T.K., Lee Y.S., Lee Y.H., Kim S., Yun H.Y. 2001. Anticarcinogenic effect of *Panax ginseng* C.A. Meyer and identification of active compounds // J. Korean Med. Sci. Vol.16 (Suppl.). P.6–18.