REFERENCES

1. I. V. Devoino and R. Yu. Il'yuchenok, in: *Monoaminerdic Systems in the Control of the Immune Response (Serotonin, Dopamine)* [in Russian], Novosibirsk (1983).
2. T. M. Ivanova, R. Kvetnanskii, T. I. Belova, et al., *Fiziol. Zh. SSSR*, № 7, 823-828 (1985).
3. G. V. Idova, I. O. Beletskaya, and L. V. Devoino, *Ibid.*, № 6, 865-870 (1988).
4. S. Arunasmitha, C. Andrade, and N. Pradhan, *Indian J. Physiol. Pharmacol.*, **33**, № 2, 132 (1989).
5. G. C. Coztzias, S. T. Miller, A. R. Nicholson, et al., *Proc. Nat., Acad. Sci. USA*, **171**, 2466-2469 (1974).
6. N. Fabris, *J. Neurosci.*, **51**, № 3-4, 373-375 (1990).
7. C. E. Finch, *Adv. Exp.Med.*, **113**,15-29 (1978).
8. M. Ghoheum, G. Gill, P. Assahan, et al., *Immunology*, **60**, 416-465 (1987).
9. K. W. Kelley, S. Brief, H. J. Westly, et al., *Ann. New York Acad. Sci.*, **496**, 91-97 (1987).
10. S. A. Lorens, N. Hata, R. Honda, et al., *Neurobiol. Aging*, **11**, № 2, 139-150 (1990).
11. L. Nagelkeron, A. Hertrogh-Huijbregts, R. Dobber, et al., *Europ. J. Immunol.*, *21*, 273-376 (1991).
12. U. Scapagnini and B. Marchetti, in: *Neuropeptides and Immunopeptides. Messengers of the Neuroimmune Axis*, New York (1989), p.7.
13. G. Shelli, G. Passeri, and P. Sansoni, *J. Gerontol.*, **37**, № 7, 477-486 (1989).
14. A. J. Stoessl, M.T. Martin-Iverson, T. M. Barth, et al., *Brain Res.*, **495**, № 1, 20-30 (1989).
15. J. L. Venero, A Machado, and J. Cano, *Mech. Ageing Dev.*, **311**, 227-233 (1989).

ONCOLOGY

Inhibition of the Development of Experimental Tumors of the Cervix and Vagina by Tinctures from Biomass of Cultured Ginseng Cells and Its Germanium-Selective Stocks

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UDC 618.1-006-092:615.015.89

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.116, № 11, pp. 534-536, November, 1993
Original article submitted June 9, 1993

Key Words: *bioginseng; carcinogenesis of cervix and vagina*

Natural phytoadaptogens are promising candidates for remedies of prophylaxis and treatment of cancer [7]. Preparations of ginseng root have been shown to exhibit an antitumor effect both in experimental animals with transplanted tumors and in clinical practice [3,7]. In addition, ginseng root extract produces an anticarcinogenic action on the growth of lung adenoma induced by different chemical carcinogens in mice [16]. Epidemiological observations have shown that in persons regu-

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0007-4888/93/0011-1423$12.50 ©1994 Plenum Publishing Corporation

TABLE 1. Effect of Bioginseng and Its Germanium-Selective Stocks on Carcinogenesis of the Cervix and Vagina

Group	Agent	Number of mice in group	Number of mice with tumors of cervix and vagina[a]		Number of mice dying from carcinomas
			carcinomas[b]	papillomas	
1.	DMBA – control (water, perorally)	38	18 (47%)	8 (21%)	17 (45%)
2.	DMBA + BG (perorally)	40	15 (37%)	5 (12%)	13 (32%)
3.	DMBA + BG–5 (perorally)	35	17 (49%)	5 (14%)	15 (43%)
4.	DMBA + BG–13 (perorally)	38	13 (39%)	6 (18%)	11 (33%)
5.	DMBA – control (saline, local application)	38	28 (74%)	4 (10%)	25 (66%)
6.	DMBA + BG (local application)	39	19 (49%)*	3 (8%)	12 (31%)*
7.	DMBA + BG–5 (local application)	21	8 (38%)*	2 (9%)	4 (19%)*
8.	DMBA + BG–13 (local application)	21	9 (43%)*	4 (19%)	5 (24%)*
9.	Intact control	20	–	–	–

Note. [a]) The data on breast adenocarcinomas and lung adenomas recorded in some cases are not presented in the table. [b]) Histologically, most carcinomas were pavement-cell cancers; in isolated cases there were adenosquamous cancers or adenocarcinomas. *: the difference in comparison with the control (group 5) is statistically significant, $p<0.001-0.05$.

larly including ginseng in their diet the risk of developing cancer of different localizations decreases [17]. Taking into account the low toxicity of ginseng, it seems fruitful to further studies on the oncoprophylactic or anticarcinogenic properties of this medicinal plant. The present study was aimed at comparing the anticarcinogenic activity of three cultural stocks of ginseng obtained by biotechnological methods.

MATERIALS AND METHODS

The experiments were carried out on female SHR mice (provided by the Rappolovo nursery, Russian Academy of Medical Sciences). The carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) was from Sigma, USA. Ginseng cell cultures (bioginseng, BG, and BG-5 and BG-13) in the form of 40% ethanol tinctures were obtained from the Kirishi Biochemical Plant, St. Petersburg Region. BG and its two germanium-selective stocks BG-5 and BG-13 were produced by cultivating cells of ginseng (*Panax ginseng C. A. Mey*) root in a conventional medium [2] or in media containing organogermanium compounds 1-hydroxygermatran and 2-carboxyethyl- germsesquioquioxan, respectively [5]. Every day before use of the drug, ethanol was evaporated in a vacuum-rotary evaporator and water was added to the initial volume.

Squamous-cell carcinomas of the cervix and vagina were induced in mice by intravaginal applications of 0.1% DMBA solution in triethyleneglycol on polyurethane tampons, the procedure being repeated twice a week during 6 weeks (the summary DMBA dose constituted 300 μg per mouse). After completion of the DMBA treatment and up to the end of observations in the first series of experiments the control mice (1st group) received water and those of the 2nd, 3rd and 4th groups were given BG, BG-5, and BG-13 solutions, respectively. The preparations were administered perorally with a probe, five days a week with a 2-day interval in a dose of 0.15 ml per mouse per day (7.5 ml/kg body weight). In the second series of experiments local applications of polyurethane tampons soaked in either physiological solution (control group 5) or aqueous solutions of BG, BG-5, and BG-13 in groups 6, 7, and 8, respectively, were used. Vaginal insertions of tampons were repeated during the postinitiation period twice a week till the end of observation. The drug dose constituted 30 μl per application. In the 9th group the animals were not subjected to any treatment at all (intact control). The surviving mice were killed with ether fumes 22 weeks after the beginning of DMBA initiation in groups 5-8 and after 44 weeks in groups 1-4 and 9. All dying and sacrificed animals were subjected to full autopsy; the organs with tumors were treated histologically according to a conventional protocol using hematoxylin/eosin staining and analyzed by light microscopy. The data obtained were subjected to statistical analysis using the χ^2 test.

RESULTS

In all groups of mice the carcinogen DMBA induced predo- minantly malignant (only in 8-21% of cases, benign) tumors of the cervix and vagina (Table 1). No tumors of this localization developed spontaneously (9th group). When used perorally, the ginseng preparations BG and BG-13 exhibited only an insignificant tendency to inhibit carcinogenesis of the cervix and vagina and to reduce mortality in comparison with the control group; BG-5 had no influence on tumor growth at all.

In the control group 5, cervical and vaginal tumors developed more rapidly than in group 1. This difference may be associated with the promoting effect of the vaginal tampons during the postinitiation period due to constant local irritation of the target tissues and development of an inflammatory reaction [8]. As follows from Table 1, all three drugs tested in local applications lowered the frequency of induced cervical and vaginal carcinomas and reduced mortality from the disease: BG by 25 and 35%, BG-5 by 36 and 47%, and BG-13 by 31 and 42%, respectively.

Thus, BG and its germanium-selective stocks in local application effectively inhibited carcinogenesis of the cervix and vagina, while when used perorally they exhibited only an insignificant tendency to inhibit tumor development.

Ginseng preparations are known to possess adaptogenic properties [2,7] and an immunostimulating activity (activation of natural killers, stimulation of macrophage reaction, interferon properties) [6,7,9]. Ginsengosides exhibit a cytodifferentiating activity in respect to different tumor cell lines [13,14] and produce antioxidant [12] and antiinflammatory effects [11]. The ability to increase the cAMP level in the cell was revealed for ginseng polysaccharides [15]. All these mechanisms are engaged and play an important role in the inhibition of tumor growth. It is most likely that not one but various mechanisms underlie the revealed anticarcinogenic effects of bioginseng preparations. Organogermanium compounds used for the production of BG-5 and BG-13 possess an antitumor and anticarcinogenic activity [4,10]. However, the direction and strength of such effects of the three bioginseng stocks with respect to experimental tumors of the cervix and vagina proved to be similar.

On the whole, the results of this study testify that application of BG and its germanium-selective stocks as local agents in the treatment of precancerous diseases and prophylaxis of cervical cancer may have good prospects after thorough clinical investigations. Such a conclusion acquires particular importance in view of the high incidence of precancerous diseases of the cervix and the lack of effective conservative means for treating this pathology [1].

REFERENCES

1. Ya. V. Bokhman and U. K. Lyutra, *Cancer of the Cervix* [in Russian], Kishinev (1991).
2. A. N. Kudrin, F. P. Krendal', I. K. Sokolov, *et al.*, *Farmatsiya*, **31**, № 5, 33-38 (1982).
3. N. V. Lazarev, *Vopr. Onkol.*, **11**, № 12, 48-54 (1965).
4. E. Ya. Lukevits, T. K. Gar, L. M. Ignatovich, and V. F. Mironov, *Biological Activity of Germanium Compounds* [in Russian], Riga (1990).
5. L. I. Slepyan, Zh. P. Graudine, L. E. Poluektova, *et al.*, in: *Natural Immunomodulators. Synopses of Reports of Conference* [in Russian], Vladivostok (1990).
6. V. N. Chubarev, E. R. Rubtsova, I. V. Filatova, *et al.*, *Farmakol. Toksikol.*, **52**, № 2, 55-69 (1989).
7. K. V. Yaremenko, *Adaptogens as Agents of Preventive Medicine* [in Russian], Tomsk (1990).
8. P. A. Cerutti, *Carcinogenesis*, **9**, 519-526 (1989).
9. P. Devaleou, P. Lallouette, and A. M. Tessier, *Planta Med.*, **46**, 49-54 (1980).
10. J. J. Jang, K. J. Cho, Y. S. Lee, and J. H. Bae, *Carcinogenesis*, **12**, 691-695 (1991).
11. H. Matsuda, K.-I. Samukawa, and M. Kubo, *Planta Med.*, **56**, 19-23 (1990).
12. S. Nakagawa, S. Joshida, J. Hirao, *et al.*, *Hiroshima J. Med. Sci.*, **34**, 303-309 (1985).
13. T. Ota, K. Fujikawa-Yamamoto, Z.-P. Zong, *et al.*, *Cancer Res.*, **47**, 3863-3867 (1987).
14. K. Umehara, R. Takagi, M. Kuroyanagi, *et al.*, *Chem.-Pharm. Bull.*, **40**, 401-405 (1992).
15. M. Yang and B.-X. Wang, *Acta Pharmacol. Sin.*, **12**, 272-275 (1991).
16. T. K. Yun, *J. Toxicol. Sci.*, **16**, Suppl. I, 53-62 (1991).
17. T. K. Yun and S. Y. Choi, *Int. J. Epidemiol.*, **19**, 871-876 (1990).