DOI 10.1007/s10517-017-3904-x
Bulletin of Experimental Biology and Medicine, Vol. 163, No. 6, October, 2017 ONCOLOGY

ONCOLOGY

Effect of Phytoadaptogen Administration during Early Ontogeny on Lifespan and Somatic Status of CBA Mice with High Incidence of Tumors

E. V. Bocharov[1], R. V. Karpova[1], O. A. Bocharova[1], V. G. Kucheryanu[2], and Z. S. Shprakh[1]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 6, pp. 755-758, June, 2017
Original article submitted December 22, 2016

We studied the influence of nontoxic phytoadaptogen complex on the lifespan and somatic status (body weight, coat state, and motor activity) of CBA mice predisposed to spontaneous hepatomas. Administration of the complex phytoadaptogen during the first month of postnatal ontogeny increased mean animal lifespan by 17.1% ($p<0.001$) and median of survival by 25.6% ($p<0.001$) and promoted maintenance of satisfactory physical status of CBA mice during spontaneous hepatocarcinogenesis.

Key Words: *hepatocarcinogenesis; survival; phytoadaptogens; ginseng; Rhodiola rosea*

Preventive measures against malignant neoplasms can be directed at regulation of adhesion cell cooperation, because disturbances of cell—cell adhesion can be a key mechanism of the tumor process [5]. Phytoadaptogens capable of normalizing intercellular adhesion and improving immunological reactivity of the organism against tumors are of particular interest.

In preventive oncology, the preventive effect of drugs is assessed by modulation of spontaneous tumor formation in linear mice. CBA mice characterized by high incidence of hepatomas represent a classical experimental model of spontaneous carcinogenesis. In male CBA mice, the first hepatomas develop at the age of 6 months and by the age of 18 to 22 months, the tumors are detected in 100% mice [14].

Previous studies have demonstrated that phytoadaptogen complex (PAC) administered to CBA male mice predisposed to spontaneous hepatocarcinomas during the early postnatal ontogeny regulated cell—cell interactions, stably increased expression of leukocyte integrins LFA-1 and Mac-1, and reduced serum level of suppressor cytokines IL-6 and IL-10 [1]. The number and size of spontaneous hepatocarcinomas in PAC-treated mice were lower than in the control [6]. At the same time, morphological analysis revealed leukocytic (primarily lymphocytic) infiltration and destruction of hepatocarcinomas that by the age of 22 months were presented by low-differentiated trabecular-acinar hepatomas [2].

Here we studied the effect of PAC administered during the early postnatal ontogeny to CBA male mice characterized by hereditary predisposition to tumor formation on their lifespan and somatic status.

MATERIALS AND METHODS

CBA male mice with high incidence of tumor formation (subline of CBA/Lac Y) were obtained from the Breeding Center of N.N. Blokhin Russian Cancer Research Center. The animals were kept under standard

[1]N. N. Blokhin Russian Cancer Research Center, Ministry of Health of the Russian Federation; [2]Research Institute of General Pathology and Pathophysiology, Moscow, Russia. ***Address for correspondence:*** planta39@rambler.ru. R. V. Karpova

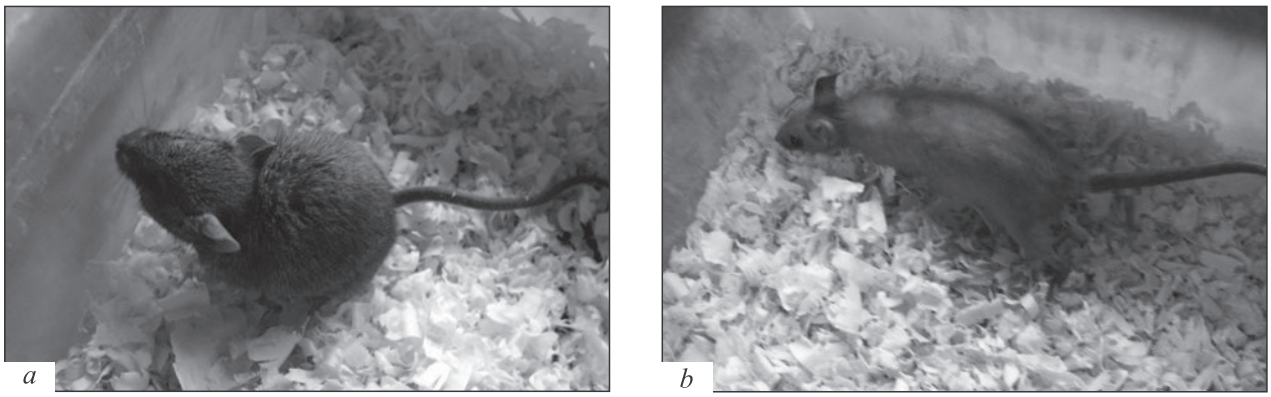

Fig. 1. Appearance of CBA mice: experimental mouse aged 33 months (*a*) and control animal aged 23 months (*b*).

vivarium conditions in accordance with international ethical norms and received standard ration. PAC (10% solution) was administered to mice of the experimental group (n=48) with drinking water during the first month of life including the period of completion of liver differentiation (5-15 day). PAC was administered to pregnant females during gestation until weaning of pups at the age of 3 weeks; then, male pups received PAC with water during 1 week.

The mice of control group 1 (n=61) received water. As PAC is a water-ethanol extract, mice of control group 2 (n=22) received 3% ethanol in water (concentration of ethanol in PAC preparation). The results in the two control groups did not differ significantly and they were combined into a common control group (n=83).

The animals were weighed at the age of 4, 8, and 22 months. During late ontogeny the state of the coat was assessed and motor activity in the open-field test was evaluated in the automatic mode with Opto-Varimex-3 system (Columbus Instruments).

PAC is a non-toxic herbal formula consisting of water-ethanol extracts of forty plant included in the State Pharmacopoeia of the Russian Federation, including known adaptogens of *Panax ginseng*, *Rhodiola rosea*, *Eleutherococcus senticosus*, *Schisandra chinensis*, and *Aralia mandshúrica*. Ginsenosides, aralosides, eleutherosides, flavonoids, polyphenols, vitamins, amino acids and other biological active components were identified in PAC and biological and chemical methods of PAC standardization were developed [8-10]. Immunomodulating, antioxidant, antimutagenic, antitumour properties of PAC were demonstrated in previous studies [3,4,7].

Statistical analysis of the results was conducted in Statistica 6.0 software by ANOVA test; statistical significance was evaluated using the Newman—Keuls criteria. Animal survival was analyzed by the Kaplan—Meier method; significance of differences between the groups was evaluated using Cox F test.

RESULTS

The body weight of mice of both groups increased by 8 months (from 30.3±0.4 to 32.2±0.5 g in the control and from 31.2±0.5 to 34.0±0.3 g in the experimental, p=0.07). At the age of 22 months, the body weight of the control animals decreased from 32.2±0.5 to 24.4±0.4 g (p=0.0001) and was lower by 29% than in experimental animals that did not lost weight during this period (34.0±0.3 and 34.6±0.3 g at the age of 8 and 22 months, respectively).

These results can attest to cachexia of varying degree in control animals determined by both age-related changes and tumor process. In controls, serum levels of IL-6 and IL-10 were increased, which contributed to an increase in serum level of C-reactive protein and cleavage of muscle proteins leading cachexia [11,12].

TABLE 1. Parameters of Motor Activity of CBA Mice during Late Ontogeny after Administration of PAC

Group	Age, month	Ambulation, cm	Time without movements, sec	Number of rearing postures	Number of small movements
Control (n=16)	23.9±0.1	1089.6±74.8	82.5±7.6	10.0±1.8	220.5±21.0
Experimental (n=7)	30.1±0.3 $p \leq 0.001$	933.7±150.2 p=0.12	109.3±16.7 p=0.07	5.5±1.3 p=0.31	195.5±20.0 p=0.02

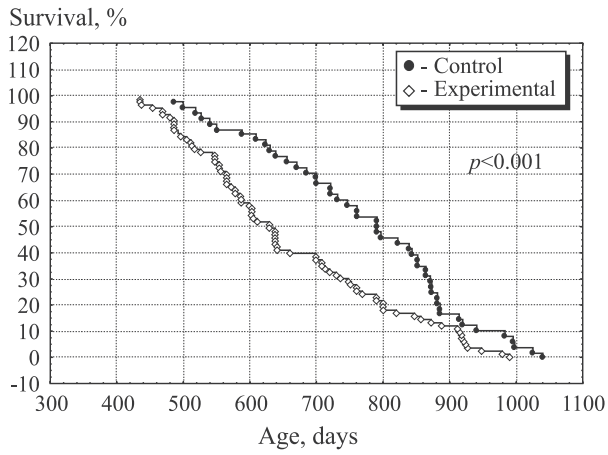

Fig. 2. Survival curves in CBA mice receiving PAC.

In animals of the experimental group, body weight did not decrease, which was associated with reduced serum levels of IL-6 and IL-10 [1]. It can be hypothesized that, PAC reduces the inflammatory reaction, prevents degradation pf proteins and, consequently, the loss of muscular mass.

Mice of the experimental group had normal coat, which was associated with a decrease in serum IL-6 level (Fig. 1, *a*). The latter can prevent hair loss via stimulation of functional activity of hair follicles under conditions of suppression of the inflammatory process in the skin [15]. In the control group, alopecia was detected in 16.7% mice (Fig. 1, *b*).

The groups did not significantly differ by motor activity in the late ontogeny (Table 1), despite the fact that control mice (23.9±0.1 months) at the time of measurement were 6-7 months younger than experimental ones (30.1±0.3 months).

As a result, the mean lifespan in the control group was 662.0±16.5 days (21.7 months) and median survival was 631.4 days (20.7 months). Administration of PAC during the first month of postnatal development led to an increase in animal lifespan by 17.1% (to 775.1±21.3 days, or 25.4 months, $p<0.001$). The median survival rate increased by 25.6% in comparison with the control group and was 26 months (793 days) ($p<0.001$; Fig. 2). In the control group, none animals survived over 1000 days. In the experimental group, 2 mice survived this period (4.2±0.3%, $p\leq0.001$). The results are of principal importance in light of lower incidence of tumor formation and lower number and volume of tumors in the group of mice treated with PAC [6].

An increase in the lifespan of mice up to 1,356 days without tumor pathologies was attained in animals maintained at low-calorie diet from the early ontogeny [13]. The mechanism of this effect includes suppression of TOR signaling involved in regulation of growth and metabolism associated with aging.

The results of our study suggest that administration of nontoxic immunomodulator PAC during the early postnatal ontogeny increased lifespan, maintained body weight, motor activity, and coat quality in tumor-predisposed CBA male mice and suppressed the development of hepatocarcinomas.

REFERENCES

1. Bocharova OA, Bocharov EV, Karpova RV, Il'enko VA, Kazeev IV, Baryshnikov AY. Integrins LFA-1 and Mac-1 and cytokines IL-6 and IL-10 in high-cancer mice under the influence of phytoadaptogen. Bull. Exp. Biol. Med. 2014;157(2):258-260.
2. Bocharov EV, Bocharova OA, Solov'ev YN, Karpova RV, Kucheryanu VG. Morphological Studies of Hepatocellular Carcinomas in Male CBA Mice with High Liability to Cancer under the Effect of Phytoadaptogen. Bull. Exp. Biol. Med. 2016;161(5):727-730.
3. Bocharova OA, Davydov MI, Klimenkov AA, Baryshnikov AY, Karpova RV, Chulkova SV, Gorozhanskaya EG, Ilyenko VA. Prospects of using phytoadaptogen in the treatment of diffuse stomach cancer. Bull. Exp. Biol. Med. 2009;148(1):82-85.
4. Bocharov EV, Ivanova-Smolenskaya IA, Poleshchuk VV, Kucheryanu VG, Il'enko VA, Bocharova OA. Therapeutic efficacy of the neuroprotective plant adaptogen in neurodegenerative disease (Parkinson's disease as an example). Bull. Exp. Biol. Med. 2010;149(6):682-684.
5. Bocharova OA, Karpova RV. Adhesion in cancer biology. Ross. Bioter. Zh. 2006;5(3):55-60. Russian.
6. Bocharova OA, Karpova RV, Bocharov EV, Solov'yov YN. Effects of Phytoadaptogen on Hepatoma Development in Cancer-Prone CBA Mice. Bull. Exp. Biol. Med. 2015;159(5):655-657.
7. Bocharova OA, Pozharitskaya MM, Chekalina TL, Voronin VF. Modern Aspects of Pathogenesis and Treatment of Leukoplakia of the Oral Mucoa. Methodical Manual for Physicians. Moscow, 2004. Russian.
8. Karpova RV, Shevchenko VE, Bocharov EV, Sheychenko OP, Bocharova OA, Kucheryanu VG, Bykov VA. Ginsenosides definition in plant extracts by means of high through liquid chromatography with tandem mass-spectrometry. Ross. Bioter. Zh. 2016;15(2):36-46. Russian.
9. Sheichenko OP, Bocharova OA, Krapivkin BA, Uyutova EV, Karpova RV, Kazeev IV, Bocharov EV, Bykov VA. HPLC Study of Multiphytoadaptogen. Vopr. Biol. Med. Farm. Khimii. 2012;(10):52-59. Russian.
10. Sheichenko OP, Bocharova OA, Sheichenko VI, Tolkachev ON, Bocharov YeV, Karpova RV, Bykov VA. A possibility of using electronic absorption spectra to standardize the multicomponent drug Phytomix-40. Vopr. Biol. Med. Farm. Khimii. 2007;5(2):20-25. Russian.
11. Deans DA, Tan BH, Ross JA, Rose-Zerilli M, Wigmore SJ, Howell WM, Grimble RF, Fearon KC. Cancer cachexia is associated with the IL10-1082 gene promoter polimorphism in patients with gastroesophageal malignancy. Am. J. Clin. Nutr. 2009;89(4):1164-1172.
12. Krzystek-Korpacka M, Matusiewicz M, Diakowska D, Grabowski K, Blachut K, Kustrzeba-Wojcicka I, Terlecki G, Gamian A. Acute-phase response proteins are related to cachexia

and accelerated angiogenesis in gastroesophageal cancers. Clin. Chem. Lab. Med. 2008;46(3):359-364.
13. Schumacher B, van der Pluijm I, Moorhouse MJ, Kosteas T, Robinson AR, Suh Y, Breit TM, van Steeg H, Niedernhofer LJ, van Ijcken W, Bartke A, Spindler SR, Hoeijmakers JH, van der Horst GT, Garinis GA. Delayed and accelerated aging share common longevity assurance mechanisms. PLoS Genet. 2008;4(8):e1000161. doi: 10.1371/journal.pgen.1000161.
14. Sharp JG, Riches AC, Littlewood V, Thomas DB. The incidence, pathology and transplantation of hepatomas in CBA mice. J. Pathol. 1976;119(4):211-220.
15. Yu M, Kissling S, Freyschmidt-Paul P, Hoffmann R, Shapiro J, McElwee KJ. Interleukin-6 cytokine family member oncostatin M is a hair-follicle-expressed factor with hair growth inhibitory properties. Exp. Dermatol. 2008;17(1):12-19.