Metformin Decelerates Aging and Development of Mammary Tumors in HER-2/neu Transgenic Mice

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Transgenic FVB/N female mice carrying HER-2/neu mammary cancer gene received metformin (1200 mg/liter) with drinking water 5 days a week starting from the age of 2 months until natural death. Metformin slightly reduced food consumption, but did not change water consumption and dynamics of weight gain. Mean life span of mice increased by 8% ($p<0.05$), in 10% long-living mice it was prolonged by 13.1%, and the maximum life span was prolonged by 1 month under the effect of metformin in comparison with the control. The rate of populational aging decreased by 2.26 times. The total incidence of mammary adenocarcinoma and their multiplicity did not change under the effect of metformin, while the latency of tumor development increased and the mean diameter of tumors decreased. Hence, we first demonstrated a geroprotective effect of metformin and its suppressive effect towards the development of mammary tumors.

Key Words: *metformin; life span; breast cancer; transgenic mice; HER-2/neu*

Caloric restriction (CR) is the most effective method for life span prolongation in laboratory animals [1,3, 11]. This diet is associated with a stable decrease in the levels plasma glucose, insulin, and many other hormones, which gave grounds to call this method "pseudohypophysectomy", because of its similar effects. On the basis of these data and observations indicating that hyperglycemia and hyperinsulinemia are destructive for the organism, it was hypothesized that the geroprotective effect of CR was explained by this mechanism [11]. However it is obvious that CR is hardly a perspective method for prolongation of the life span of humans.

Great recent attention was paid to the search for CR mimetics [1,14]. Antidiabetic biguanides seem to be the most promising in this respect. Drugs of this group (fenformin, buformin, metformin), along with hypoglycemic effect, improve glucose utilization in tissues, reduce utilization of fatty acids as energy substrates, suppress neoglucogenesis, lower blood cholesterol, triglycerides, and insulin concentrations and biosynthesis of cholesterol, and reduce body weight. These effects of antidiabetic biguanides and their capacity to eliminate signs of metabolic immunosuppression prompted their use in oncological practice for normalization of some metabolic disorders characteristic of cancer patients [2,5,9,10,12]. It was previously shown that long-term treatment with fenformine and buformine prolonged life span of nematodes [6], mice, and rats and reduced the incidence of spontaneous tumors and tumors induced by chemical carcinogens or ionizing radiation [1,8,9,13]. Considering the role of insulin in the pathogenesis of malignant tumors these data are of particular importance [10,12]. Metformin (siofor) is now widely used in clinical practice. However, preliminary data on its geroprotective activity just appeared [13].

We studied the effect of metformin on biological age, life span, and development of tumors in female mice carrying HER-2/neu breast cancer gene.

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MATERIALS AND METHODS

The study was carried out on homozygotic transgenic gene-carrying FVB/N mice (Charles River, Hollister), a gracious gift from Italian National Research Center of Aging; mouse strain was maintained at Department of Carcinogenesis and Oncogerontology, N. N. Petrov Institute of Oncology. The mice were kept at 12-h day/night regimen and 22±2°C on standard fodder with free access to water. Experiments were carried out on 66 transgenic females randomly divided into 2 groups. Starting from the age of 2 months the animals of one group received 240 mg/kg metformin (siofor, Berlin-Chemie, Menarini Group) with drinking water 5 days a week until natural death. Metformin was dissolved in tap water to a concentration of 1200 mg/liter. Fresh solution was prepared 3 times a week. The animals were weighed daily and the volumes of consumed fodder and water were evaluated. The development of mammary tumors was controlled weekly by palpation and the location and size of tumors were recorded. Dead animals were autopsied, macro- and microscopic studies were carried out. The results of experiments were statistically processed using Fisher's precise test, Wilcoxon—Mann—Whitney test, Student—Newman—Cools test, and ANOVA analysis [4]. Survival was analyzed by Cox method [7].

RESULTS

Injection of metformin somewhat decreased fodder consumption, but not drinking, and did not influence the dynamics of weight gain. The mean life span (MLS) of mice increased by 8% ($p<0.05$) under the effect of the drug, in 10% long-living animals it was prolonged by 13.1%, and the maximum life span was 1 month longer than in the control (Table 1). The rate of populational aging in mice (constant α in Hompertz equation) decreased 2.26 times ($p<0.05$) under the effect of metformin, while mouse survival curve shifted appreciably to the right under the effect of metformin (Fig. 1, *a*).

The dynamics of mammary tumor development was virtually the same in both groups until month 5 of life, after which a clear-cut deceleration of tumor development was noted in the experimental group (Fig. 1, *b*). The incidence of mammary adenocarcinomas in mice carrying HER-2/neu gene was 100% in both groups. The incidence, multiplicity, and incidence of mammary tumor metastases in the lung also virtually did not differ in the groups. The percentage of mice with 4-6 tumors in the metformin group was virtually the same as in the control group (8.9 and 9.3%, respectively), while the percentage of mice with 9 or 10 tumors decreased 2-fold under the effect of metformin in comparison with the control (46.9 and 23.5%, respectively, $p<0.05$). The size of mammary adenocarcinoma under the effect of the drug was also somewhat lower ($p<0.05$).

Breast cancer is one of the most prevalent malignant tumors and the leading cause of death from cancer in women [2]. Transgenic mice carrying HER-2/neu gene belonging to the epidermal growth factor tyrosine kinase receptors (EGFR) are characterized by high incidence of mammary tumors and short life span [1]. Our experiments demonstrate deceleration of aging and development of breast tumors in this mouse strain under the effect of metformin. Experiments on long-living rats and mice resistant and sensitive to cancer

TABLE 1. Metformin Effect on the Life Span and Development of Mammary Adenocarcinoma in HER-2/neu Transgenic Mouse Females

Parameter		Control	Metformin
Number of mice		34	32
Life duration, days	mean	264.0±3.5	285.0±5.2+
	maximum	311	340
Mean life span of 10% long living mice, days		297.0±7.3	336.0±2.7+
α, day^{-1}*		0.0762	0.0337+
Number of mice of mammary tumors		34 (100%)	32 (100%)
Day of detection of the first tumor		135	128
Mean latent period of mammary tumor, days		174.0±2.4	187.0±3.5+
Total number of tumors		290	263
Mean number of tumor per mouse in the group		8.50±0.25	8.20±0.23
Mean diameter of tumors, cm		1.790±0.055	1.590±0.056+
Number of mice with metastases in the lung		24 (71%)	23 (72%)

Note. *Constant a in Hompertz equation $R=R_0(exp)\alpha t$, where R_0 is mortality during $t=0$. *$p<0.05$ compared to the control.

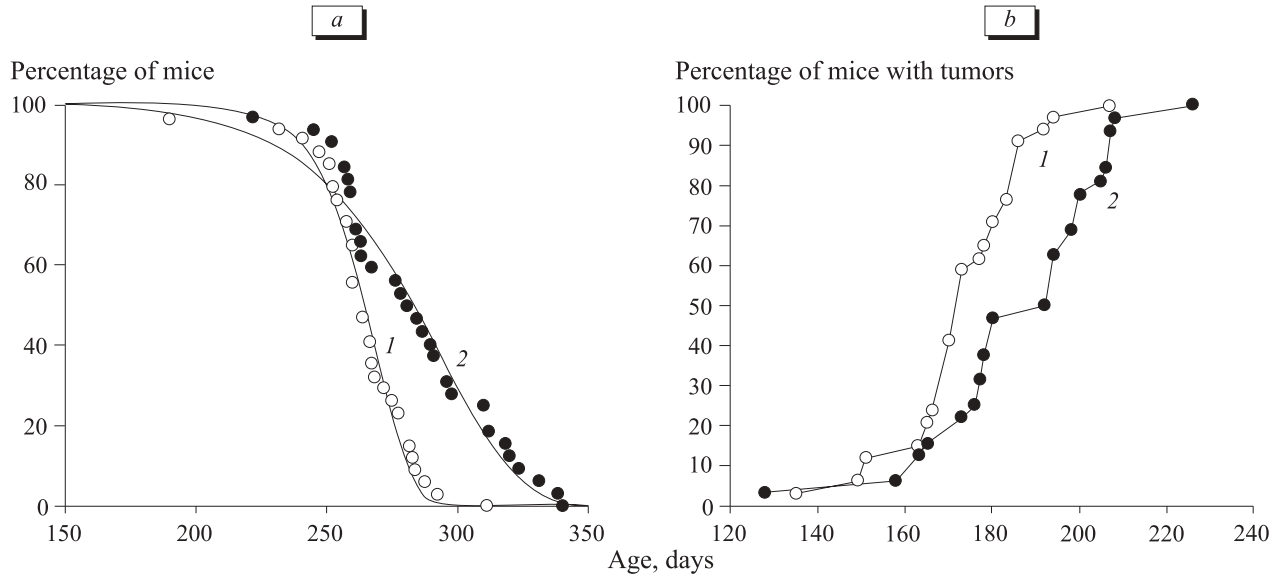

Fig. 1. Effect of metformin on life span and development of mammary adenocarcinoma in HER-2/neu transgenic female mice. *a*) dynamics of mouse survival; *b*) dynamics of development of new tumors. *1*) control; *2*) metformin.

showed that other biguanides, *e.g.* fenformine and buformine, were characterized by geroprotective and anticarcinogenic effects [1,5,8,9]. The data on the antioxidant effects of antidiabetic biguanides, their direct effect on the mitochondria, and neuroprotective activity recommend biguanides for the prevention of neurodegenerative diseases [1-3]. Metformin modulated activities of genes whose expression changed due to CR [13]. Hence, we revealed a geroprotective effect of metformin in mice and its inhibitory effect on the development of tumors, caused by expression of HER-2/neu oncogene. It will be interesting to evaluate metformin efficiency on other, *e. g.* cancer-resistant animals.

REFERENCES

1. V. N. Anisimov, *Molecular and Physiological Mechanisms of Aging* [in Russian], St. Petersburg (2003).
2. L. M. Bershtein, *Oncoendocrinology. Traditions, State of the Art, Prospects* [in Russian], St. Petersburg (2004).
3. A. I. Bozhkov, *Uspekhi Gerontol.*, **8**, 89-99 (2001).
4. E. V. Gubler, *Computation Methods of Analysis and Recognition of Pathological Processes* [in Russian], Leningrad (1978).
5. V. N. Anisimov, *Exp. Gerontol.*, **38**, No. 10, 1041-1049 (2003).
6. V. V. Bakaev, *Biogerontology*, **3**, Suppl. 1, 23-24 (2002).
7. D. R. Cox and D. Oakes, *Analysis of Survival Data*, London (1996).
8. V. M. Dilman and V. N. Anisimov, *Gerontology*, **26**, No. 5, 241-245 (1980).
9. V. M. Dilman, L. M. Bershtein, M. A. Zabezhinski, *et al.*, *Arch. Geschwulforsch.*, **48**, No. 1, 1-8 (1978).
10. F. S. Facchini, N. W. Hua, G. M. Reaven, and R. A. Stoohs, *Free Radic. Biol. Med.*, **29**, No. 12, 1302-1306 (2000).
11. E. J. Masoro, *Exp. Gerontol.*, **35**, 299-305 (2000).
12. M. N. Pollak, E. S. Schernhammer, and S. E. Hankinson, *Nat. Rev. Cancer*, **4**, No. 7, 505-518 (2004).
13. S. R. Spindler, J. D. Dhahbi, P. L. Mote, *et al.*, *Biogerontology*, **4**, Suppl. 1, 89 (2003).
14. R. Weindruch, K. P. Keenan, J. M. Carney, *et al.*, *J. Gerontol. Biol. Sci.*, **56 A**, Special Issue 1, 20-33 (2001).