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Insulin in aging and cancer: antidiabetic drug diabenol as geroprotector and anticarcinogen

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Abstract

The effects of new antidiabetic drug Diabenol® (9-β-diethylaminoethyl-2,3-dihydroimidazo-(1,2-α)benzimidazol dihydrochloride) on life span and spontaneous tumor incidence in NMRI and transgenic HER-2/neu mice as well as on colon carcinogenesis induced by 1,2-dimethylhydrazine in rats are studied. It is shown that treatment with the drug failed influence body weight gain dynamics, food and water consumption and the body temperature, slowed down age-related disturbances in estrous function and increased life span of all and 10% most long-living NMRI mice. The treatment with diabenol inhibited spontaneous tumor incidence and increased the mammary tumor latency in these mice. Diabenol treatment slowed down age-related changes in estrous function in HER-2/neu mice, failed influence survival of these mice and slightly inhibited the incidence and decreased the size of mammary adenocarcinoma metastases into the lung. In rats exposed to 1,2-dimethylhydrazine, treatment with diabenol significantly inhibited multiplicity of all colon tumors, decreased by 2.2 times the incidence of carcinomas in ascending colon and by 3.1 times their multiplicity. Treatment with diabenol was followed by higher incidence of exophytic and well-differentiated colon tumors as compared with the control rats exposed to the carcinogen alone (76.3% and 50%, and 47.4% and 14.7%, respectively). Thus, the drug increases survival and inhibits spontaneous carcinogenesis in mice and inhibits colon carcinogenesis in rats.

Keywords: Diabenol; Antidiabetic drugs; Aging; 1,2-Dimethylhydrazine; Mammary carcinoma; Colon cancer; Carcinogenesis

1. Introduction

The potential link between aging and insulin/IGF-1 signaling has attracted substantial attention during last

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years, on the basis of evidence including age-related increase in incidence of insulin resistance and type 2 diabetes in accelerated aging syndromes as well as life span extension by caloric restriction (CR) in rodents. Concomitant reduction in plasma insulin and plasma glucose levels, which implies increases sensitivity to insulin, emerges as a hallmark of increased longevity (Bartke et al., 2003; Tatar, Bartke, & Antebi, 2003). Hyperglycemia is an important aging factor involved in generation of advanced glycosylation endproducts (AGEs) (Elahi et al., 2002; Facchini, Hua, Reaven, & Stoohs, 2000). There is evidence that hyperinsulinemia favors accumulation of oxidized protein by reducing its degradation as well as facilitates protein oxidation by increasing steady-state level of oxidative stress (Facchini et al., 2000; Xu & Bard, 1999). Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, obesity, cataracts, vascular and microvascular damage (Dilman, 1994). It is important to stress that hyperinsulinemia is an important factor not only in aging but also in the development of cancer (Colangelo, Gapstur, Gann, Dyer, & Liu, 2002; Dilman, 1978, 1994; Gupta, Krishnaswamy, Karnad, & Peiris, 2002; Pollak, Schernhammer, & Hankinson, 2004).

In organisms ranging from yeast to rodents, both calorie restriction and mutations in insulin/IGF-1 signaling pathway extend life span (Bartke et al., 2003; Chiba, Yamaza, Higami, & Shimokawa, 2002; Koubova & Guarente, 2003; Longo & Finch, 2003; Roth, Ingram, & Lane, 1999; Tatar et al., 2003; Weindruch & Walford, 1988). Both approaches have some side effects. For example, calorie restriction increases the level of serum glucocorticoids and decreases resistance to infection (Masoro, 2000, 2003; Sun, Muthukumar, Lawrence, & Fernandes, 2001) whereas genetic modifications on insulin/IGF-1 signaling pathway cause obesity, dwarfism and cardiopulmonary lesions (Longo & Finch, 2003). Reviewing the available data on the benefits and adverse effects of calorie restriction and genetic modifications, Longo and Finch (2003) suggested three categories of drugs which may have potential to prevent or postpone age-related diseases and extend life span: drugs that (1) stimulate dwarf mutations, and therefore, decrease pituitary production of GH; (2) prevent IGF-1 release from the liver; or (3) decrease IGF-1 signaling by the action on either extracellular or intracellular targets.

The concept of CR mimetics is now being intensively explored (Hadley et al., 2001; Mattson et al., 2001; Weindruch et al., 2001). CR mimetics involve interventions that produce physiological and anti-aging effects similar to CR. It was suggested to use biguanide antidiabetics as a potential anti-aging treatment (Anisimov, 2003; Anisimov, Semenchenko, & Yashin, 2003; Dilman, 1971, 1978; Dilman & Anisimov, 1980). The antidiabetic drugs, phenformin, buformin, and metformin, were observed to reduce hyperglycemia and produce the following effects: improved glucose utilization; reduced free fatty acid utilization, gluconeogenesis, serum lipids, insulin and IGF-1, and reduced body weight both in humans and experimental animals (Dilman, 1994). There are no available data on the effect of other than biguanides antidiabetic drugs on life span of animals.

Diabenol® (9-β-diethylaminoethyl-2,3-dihydroimidazo-(1,2-α)benzimidazol dihydrochloride) was synthesised in Rostov State University, and its hypoglycemic activity was evaluated as 1.5 times more effective than of maninil (glibenclamide) and equal to the effect of glyclazide (pioglitazone) in rats, rabbits and dogs (Anisimova et al., 2002; Spasov, Dudchenko, & Gavrilova, 1997). It was shown that hypoglycemic effect of diabenol included both pancreatotropic and extrapancreatic pathways. Diabenol restores physiological profile of insulin secretion and decreases tissue resistance to insulin, prolongs hypoglycemic effect of insulin. It increases glucose utilization in glucose loading test in the old obese rats. It was suggested that diabenol influence insulin receptors in peripheral tissues. Diabenol increases uptake of glucose by isolated rat diaphragm in vitro both without supplementation of insulin into the medium or with supplemented insulin. Diabenol also decreases platelet and erythrocyte aggregation and blood viscosity, inhibits mutagenic effect of 2-acetylaminofluorene and has antioxidant activity (Anisimova et al., 2002; Mezheritski et al., 1998; Spasov, Ostrovskii, Ivakhnenko, Kosolapov, & Anisimova, 1999; Zinovieva, Ostrovskii, Anisimova, & Spasov, 2003). Thus, these results suggest that like biguanides diabenol has a potential to increase the life span and inhibit carcinogenesis.

In this paper, we present the results of experiments with diabenol on some ageing related biological parameters, survival and spontaneous tumorigenesis in female NMRI and transgenic HER-2/neu mice as well as

on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats.

2. Material and methods

2.1. Animals

One hundred female NMRI 2-month-old mice and 39 female 2-month-old LIO rats (Anisimov et al., 1989) were obtained from the Animal Department of N.N. Petrov Research Institute of Oncology. Homozygous *HER*-2/*neu* transgenic mice obtained from Charles River (Hollister, CA) by the Italian National Research Center for Aging were housed and breed in the Laboratory of Carcinogenesis and Aging. The mice were kept 5–7 in polypropylene cages (30 cm × 21 cm × 10 cm) and rats were kept 5 in cages (46 cm × 32 cm × 16 cm) under standard light/dark regimen (12 h light:12 h darkness) at temperature 22 ± 2 °C and received standard laboratory chow (Anisimov et al., 2002) and tap water ad libitum.

Animals were checked daily by animal care personnel and weekly by a veterinarian. The study was carried out in accordance with the regulations for ensuring the humane treatment of animals under the approval of the Committee on Animal Research of the N.N. Petrov Research Institute of Oncology.

2.2. Chemicals

Diabenol, 100% pure, was provided by Dr. V.A. Anisimova, Ph.D., Physical Chemistry Research Institute, Rostov-on-Don, Russia (Anisimova et al., 2002).

1,2-Dimethylhydrazine dihydrochloride (DMH) was provided by Sigma (USA), and was kept at −20 °C.

2.3. Experiment

2.3.1. NMRI mice

At the age of 2 months, NMRI mice were randomly divided into 2 groups, 50 animals in each, and they were individually marked. One group of mice were given diabenol in drinking water (0.1 mg/ml) 5 days a week monthly until their natural deaths. Control mice were given tap water without diabenol. Fresh solution was prepared ex tempore three times a week.

Once every 3 months, simultaneously with weighing, the amount of drinking water and consumed food was measured and the rate of consumed food (grams) per mouse was calculated.

Once every 3 months, vaginal smears, taken daily for 2 weeks from the animals, were cytologically examined to estimate the phases of their estrous functions. In the same period, rectal body temperatures of the mice were measured with an electronic thermometer, TPEM (KMIZ, Russia). The animals were observed until their natural deaths. The date of each death was registered, and the mean life span, the age at which 90% of the animals died, and the maximum life span were estimated.

2.3.2. HER-2/neu mice

At the age of 2 months, HER-2/neu 57 mice were randomly divided into two groups. All mice were individually marked. One group of 28 mice were given diabenol in drinking water (0.1 mg/ml) 5 days a week monthly until their natural deaths. Twenty-nine control mice were given tap water without diabenol. Fresh solution was prepared ex tempore three times a week. The observation schedule and tests were the similar to these in experiments with NMRI mice.

2.3.3. LIO rats

Thirty-nine 3-month-old outbred LIO rats were randomly subdivided into two groups. All rats were exposed weekly to five subcutaneous injections of DMH at a single dose of 21 mg/kg of body weight (calculated as a base). In this regimen, the carcinogen induced colon tumors in the majority of rats (Pozharisski, Likhachev, Klimashevski, & Shaposhnikov, 1979). DMH was ex tempore dissolved in normal saline and neutralized with sodium bicarbonate (pH 7.0). Additionally, 5 days a week, 20 rats of one group were given diabenol with tap water (0.1 mg/ml) starting from the day of the first injection of the carcinogen during 26 weeks whereas 19 rats of another group were not given the drug. The experiment was finalized 6 months after the first injection of the carcinogen, and all rats were sacrificied by ether vapor.

2.4. Pathomorphological examination

All the animals that died or that were sacrificed when moribund were autopsied. At autopsy their skin and all internal organs were examined. Revealed neopla-

sia were classified according to the recommendations of the International Agency of Research on Cancer (IARC) as "fatal" (i.e., those, that directly caused the death of the animal) or as "incidental" (for the cases in which the animal died of a different cause) (Gart, Krewski, Lee, Tarone, & Wahrendorf, 1986). All the tumors, as well as the tissues and organs with suspected tumor development, were excised and fixed in 10% neutral formalin. In rats, intestines were opened longitudinally. The position and size of each tumor were recorded on special charts (Pozharisski, 1990). After routine histological processing, the tissues were embedded in paraffin. Thin, 5–7 μm histological sections were stained with hematoxylin–eosine and were microscopically examined; regarding the experimental group to which the mice belonged this was a blind process. Tumors were classified in accordance with IARC recommendations (Turusov & Mohr, 1994).

2.5. Statistics

Experimental results were statistically processed by the methods of variation statistics (Goubler, 1978). The significance of the discrepancies was defined according to Student's *t*-criterion, Fischer's exact method, a chi-square analysis, and a non-parametric criterion of Wilcoxon–Mann–Whitney (Goubler, 1978). For discrepancies in neoplasm incidence to be estimated, an IARC method of combined contingency tables calculated individually for the fatal and incidental tumors (Gart et al., 1986) as well as a prevalence analysis (McKnight & Crowley, 1984) were applied.

For survival and risk analysis, Cox's method (Cox & Oakes, 1996) was used; for testing two groups survival equality, Taron's life table test (Taron, 1975) was used. All reported values for survival tests are two sided.

2.6. Mathematical models and estimations

The mathematical model was used to describe survival under the treatment. The model is the traditional Gompertz model with survival function

$$S(x) = \exp\left\{-\frac{\beta}{\alpha}\left[\exp(\alpha x) - 1\right]\right\}$$

where parameters α and β are associated with the aging rate and the initial mortality rate, respectively. Parameter α is often characterized by the value of mortality rate doubling time (MRDT), calculated as $\ln(2)/\alpha$. Parameters for the model were estimated from empirical data by use of the maximum likelihood method implemented in the Gauss statistical system (Gauss System, 1994). Confidence intervals for the aging rate parameter estimates were calculated by profiling the log-likelihood function (Cox & Oakes, 1996).

3. Results

3.1. Experiment with female NMRI mice

3.1.1. Age-related body weight dynamics

The body weight gain dynamics was studied in the control and treated with diabenol groups of mice. It was shown that the body weight of the mice in both groups increased with age, exceeding by 12 months the body weight of 3-month-old animals by 17.6% in the control group, and by 18.6% in the group given diabenol. There were no differences in the mean body weight of mice exposed and non-exposed to the drug during the all period of observation (data not shown).

3.1.2. Age-related dynamics of food and water consumption

Regular measurements have shown that the amount of food and water daily consumed by the mice in the control group and treated with diabenol group were practically the same during the all period of observation and varied from 4.9 ± 0.40 to 5.9 ± 0.21 g/mouse of food in control group and from 4.8 ± 0.51 to 5.7 ± 0.17 g/mouse of food in diabenol-treated mice whereas water consumption varied from 4.3 ± 0.33 to 5.3 ± 0.23 ml/mouse in the controls and from 4.0 ± 0.36 to 5.2 ± 0.43 ml/mouse in mice given diabenol.

3.1.3. Age-related dynamics of estrous function in mice

Investigations of the estrous function in the animals of both groups were performed every 3 months, starting when the mice were 3 months of age. The following parameters of estrous function were estimated: the length of the estrous cycle, the relative rate of estrous cycle

Table 1
Effect of diabenol on age-related dynamics of estrus functional parameters in NMRI mice

Age (months)	No. of mice	Length of estrous cycle (days)	Rate of estrous cycles of various length (%)			Fraction of mice with regular cycles (%)	Fraction of mice with irregular cycles (%)
			<5 days	5–7 days	>7 days		
Control							
3	30	6.7 ± 0.28	17.2	44.8	38	93.5	6.5
6	28	6.2 ± 0.34	29.6	44.4	26	90	10
9	19	6.4 ± 0.63	15.8	57.9	26.3	79.2	20.8
12	18	8.0 ± 1.34	12.5	50	37.5	42.1	57.9
Diabenol							
3	30	6.4 ± 0.26	19.4	51.6	29	93.9	6.1
6	26	5.8 ± 0.38#	17.4	65.2	17.4	85.2	14.8
9	22	6.3 ± 0.44	15.8	57.9	26.3	86.4	13.6
12	22	6.1 ± 0.54**	17.6	64.8	17.6	77.3*	22.7*

* The difference with the controls of corresponding age in the control group is significant: $p < 0.05$ (Fischer's exact test).
** The difference with the controls of corresponding age in the control group is significant: $p < 0.01$ (Student's *t*-test).
# The difference from the parameter at the age of 3 months in the same group: $p < 0.05$.

phases (in percent); and the relative number of short (<5 days) and long (>7 days) estrous cycles. The relative number of animals with regular cycles and irregular cycles (persistent estrus and anestrus) was calculated as well. Judging by the data presented in Table 1, the length of estrous cycle in the control female NMRI mice was increased with the advance in age, whereas was not changed with age in the group treated with diabenol. In control mice, the relative number of short estrous cycles slightly decreased with age (29.6% at the age of 6 months and 12.5% at the age of 12 months), whereas in the mice exposed with diabenol, it was practically constant during the entire period of observation. In the controls of the oldest age group, irregular cycles were registered in 57.9% of animals and only in 6.5–10% at the age of 3 or 6 months. Treatment with diabenol significantly reduced a relative number of mice with irregular estrous cycles at the age of 12 months as compared with controls ($p < 0.05$).

Thus, these data suggest that the long-term administration of diabenol slows down age-related changes in estrous function.

3.1.4. Age-related dynamics of body temperature in mice

Both the control and exposed to the drug mice revealed significant decrease in body temperature with age, but only at the age of 6 months, the body temperature was decreased in diabenol-treated mice as compared with the controls: 38.6 ± 0.14 and 38.1 ± 0.19 °C, respectively, $p < 0.05$.

3.1.5. Survival and longevity of female NMRI mice

Survival rate dynamics in the mice treated and non-treated with diabenol are demonstrated in Table 2. As shown in the table, the survival rate dynamics were in general similar in both groups up to the age of 12 months. However, it is worthy to note that the mortality in the group given diabenol was decreased between the 12th and the 15th months of their life as compared to the control group (Fig. 1). Drastic increase in mortality between months 12 and 13 in control group related mainly to death of tumor-bearing animals in this period (lymphomas and metastasizing mammary carcinomas) (Table 3).

Table 2
Effect of diabenol on survival distribution in female NMRI mice

Group	No. of survivors at the age (months)												
	4	6	8	9	10	11	12	13	14	15	16	17	18
Control	50	49	43	38	37	33	28	12	6	5	2	0	0
Diabenol	50	47	46	42	42	36	29	22*	11	9	1	0	0

* The difference with the corresponding age in the control group is significant: $p < 0.01$ (Fischer's exact test).

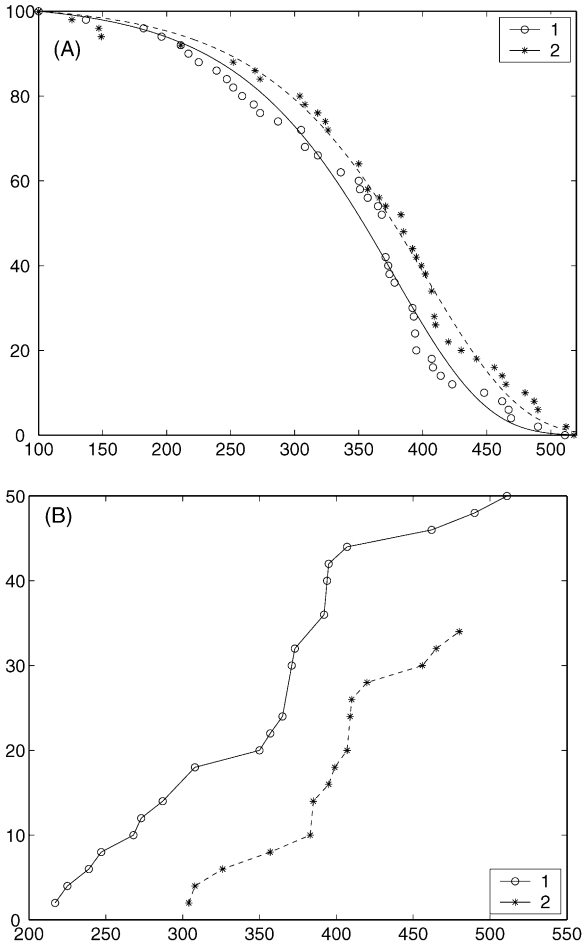

Fig. 1. Effect of diabenol on survival and tumor yield curves in female NMRI mice. (A) Ordinate, number of mice (%); abscissa, age (days); 1, control; 2, diabenol. (B) Ordinate, number of tumor-bearing mice (%); abscissa, age (days); 1, control; 2, diabenol.

Table 4
Effect of diabenol on incidence, localization and type of tumors in female NMRI mice

Parameter	Control	Diabenol
Number of mice	50	50
Number of tumor-bearing mice (%)	25 (50)	17 (34)
Age of the first mammary tumor detection (days)	176	273
Mean latency of mammary adenocarcinomas (days)	259 ± 13.8	328 ± 12.4**
Total number of mammary adenocarcinomas	26	21
Number of mammary adenocarcinoma-bearing mice (%)	21 (42)	16 (32)
Number of mammary adenocarcinomas per mouse	1.24 ± 0.1	1.31 ± 0.12
Maximum tumor size (cm)	2.51 ± 0.21	2.5 ± 0.25
Number of mice with metastases of mammary adenocarcinoma into lungs (%)	5 (10)	0*
Number of mice with malignant lymphoma	4	1

* The difference with controls is significant: $p < 0.05$ (Fischer's exact test).
** The difference with controls is significant: $p < 0.01$ (Student's *t*-test).

The mean and maximum life span of mice in the control group and in the group given diabenol was practically the same. However, the life span in the last 10% of the mice increased by the duration of diabenol treatment (by +5.0%, $p < 0.05$) (Table 3). According to the log-rank test difference between distributions of life spans in the control and experimental groups is statistically significant with *p*-value = 0.0737.

Table 3
Effect of diabenol on parameters of life span in female NMRI mice

Parameters	Control	Diabenol
Number of mice	50	50
Mean life span (days, mean ± S.E.)	346 ± 11.9	369 ± 12.9
Median (days)	371	385
Mean life span of last 10% of survivors (days)	480 ± 9.2	504 ± 6.4*
Maximum life span (days)	511	518
Ageing rate, α (days^{-1})	0.0140 (0.0139; 0.0157)	0.0136 (0.0133; 0.0145)
MRDT (days)	49.62 (44.13; 49.85)	51.09 (47.94; 51.99)

Note: Mean life span are given as mean ± standard error; 95% confidence limits are given in parentheses; MRDT, mortality rate doubling time.
* The difference with controls is significant: $p < 0.05$.

Table 5
Effect of diabenol on age-related dynamics of estrus functional parameters in HER-2/neu mice

Age (months)	No. of mice	Length of estrous cycle (days)	Rate of estrous cycles of various lengths (%)			Fraction of mice with regular cycles (%)	Fraction of mice with irregular cycles (%)
			<5 days	5–8 days	>8 days		
Control							
2	29	5.20 ± 0.18	27.5	72.5	0	100	0
5	29	5.68 ± 0.22	36.4	63.6	0	66.7	33.3
9	24	6.86 ± 0.40	14.3	57.1	28.6	80.8	19.2
Diabenol							
2	28	5.50 ± 0.29	26.5	70.6	2.9	97.1	2.9
5	28	5.31 ± 0.32	44.8	44.8	10.4	90.6*	9.4*
9	26	5.20 ± 0.22***	37.5*	60.0	2.5*	100*	0*

* The difference with the controls of corresponding age in the control group: $p < 0.05$.
*** The difference with the controls of corresponding age in the control group: $p < 0.002$ (Student's t-test).

3.1.6. Spontaneous tumor development in female NMRI mice

The total tumor incidence in the control mice was 50% (Table 4). Mammary carcinomas and malignant lymphomas developed most frequently, which corresponded to the oncological characteristics of female NMRI mice (Bomhard & Mohr, 1989). The time of the first mammary tumor detection was increased by 3 months in the group treated with diabenol, and the mean latent time of mammary tumors was increased by 2.3 months by the drug (Table 4). According to the log-rank test, difference between distributions of age of first mammary adenocarcinomas is statistically significant with p-value = 4.15×10^{-13}; difference between distributions of life spans of mice with fatal tumors (both mammary adenocarcinoma and lymphoma) is statistically significant with p-value = 0.182. The incidence of lung metastases of mammary carcinomas was 10% in the control group and 0% in the group given diabenol. In the control group, four cases of malignant lymphoma have been detected whereas in the diabenol-treated group, one case of lymphoma has been observed. The treatment with diabenol significantly shifted to right the total tumor yield curve as compared with the control group (Fig. 1).

Thus, it is worth noting that the treatment with diabenol inhibits the development of mammary carcinomas and malignant lymphomas in NMRI mice.

3.2. Experiment with female transgenic HER-2/neu mice

3.2.1. Age-related body weight dynamics

The body weight of the mice in both control and diabenol-treated groups increased with age, exceeding by 11 months the body weight of 3-month-old animals by 77.3% in the control group, and by 73.2% in the group given diabenol. There were no differences in the mean body weight of mice exposed and non-exposed to the drug during the all period of observation (data are not shown).

Table 6
Effect of diabenol on parameters of life span in female HER-2/neu mice

Parameters	Control	Diabenol
Number of mice	29	28
Mean life span (days, mean ± S.E.)	321 ± 10.4	324 ± 10.3
Median (days)	318	318
Mean life span of last 10% of survivors (days)	457 ± 21.1	444 ± 42.0
Maximum life span (days)	498	528
Aging rate, α ($days^{-1}$)	0.0307 (0.0281; 0.0337)	0.0374 (0.0351; 0.0415)*
MRDT (days)	22.58 (20.58; 24.66)	18.54 (16.71; 19.77)*

Note: Mean life spans are given as mean ± standard error; 95% confidence limits are given in parentheses; MRDT, mortality rate doubling time.
* The difference with controls is significant: $p < 0.05$.

3.2.2. Age-related dynamics of food and water consumption

The amount of food and water daily consumed by the mice in the control group and treated with diabenol group were practically the same during the all period of observation and were similar in both groups and varied from 3.27 ± 0.48 to 4.20 ± 0.14 g/mouse of food in control group and from 2.63 ± 0.14 to 4.03 ± 0.24 g/mouse of food in diabenol-treated mice whereas water consumption varied from 4.5 ± 0.51 to 5.85 ± 0.77 ml/mouse in the controls and from 4.15 ± 0.84 to 5.73 ± 0.45 ml/mouse in mice given diabenol.

3.2.3. Age-related dynamics of estrous function in mice

The length of estrous cycle in the control female HER-2/neu mice was increased with the advance in age, whereas it was not changed with age in the mice exposed to the drug (Table 5). In control mice, the relative number of short estrous cycles slightly decreased with age (36.4% at the age of 5 months and 14.3% at the age of 9 months), whereas in the mice exposed to diabenol it was practically constant during the entire period of observation. In the control group irregular cycles were registered in 33.3% of mice at the age of 5 months and in 19.2% mice at the age of 9 months. The exposure to diabenol significantly reduced a relative number of mice with irregular estrous cycles at the age of 5 and 9 months (Table 6).

Thus, these data suggest that the long-term administration of diabenol inhibits the aging of the reproductive system.

3.2.4. Age-related dynamics of body temperature in mice

Both the control and exposed to the drug mice revealed significant decrease in body temperature with age. There was no difference in average body temperature between the both groups during the entire period of observation (data are not shown).

3.2.5. Survival and longevity of female HER-2/neu mice

Survival dynamics in the mice treated and non-treated with diabenol are demonstrated at Fig. 2. The survival dynamics were in general similar in both groups during the all period of observation. According to the log-rank test life spans in the control and

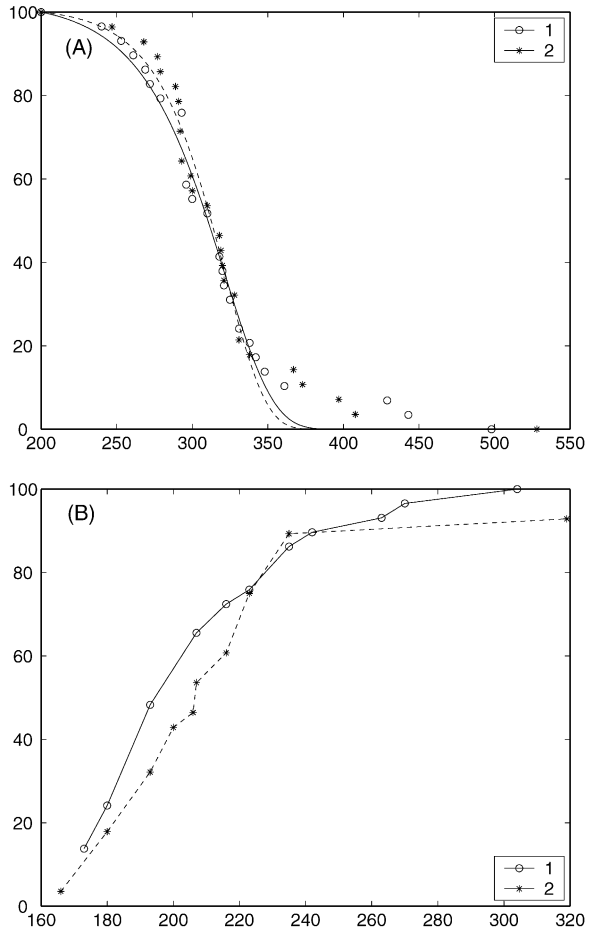

Fig. 2. Effect of diabenol on survival and tumor yield curves in female HER-2/neu mice. (A) Ordinate, number of mice (%); abscissa, age (days); 1, control; 2, diabenol. (B) Ordinate, number of tumor-bearing mice (%); abscissa, age (days); 1, control; 2, diabenol.

experimental groups are identically distributed with probability $p = 0.923$. However, maximum life span was increased by 1 months in the group treated with diabenol as compared with the controls (Table 6), although aging rate parameter α was slightly higher in diabenol-treated mice.

3.2.6. Spontaneous tumor development in female HER-2/neu mice

The incidence of mammary adenocarcinomas in the control female HER-2/neu mice was 100%. The treatment with diabenol failed influence significantly any parameter of carcinogenesis in this strain of mice. Nev-

ertheless, some parameters of spontaneous carcinogenesis have a tendency to decrease in the group treated by diabenol: total incidence of tumors (100% and 92%); total number of mammary adenocarcinoma (195 and 179), number of mice with metastases of mammary adenocarcinoma in the lung (48.3% and 35.7%), maximum size of the metastases (0.52 ± 0.11 and 0.46 ± 0.08 cm), in control and diabenol-treated group, respectively. The treatment with diabenol also slightly shifted to the right the tumor yield curve as compared with the control group (Fig. 2).

Thus, the treatment with diabenol revealed only slight tendency to the inhibition of the development of mammary carcinomas in HER-2/neu mice.

3.3. Effect of diabenol on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats

The treatment with diabenol failed influence the body weight gain, water and food consumption in rats exposed to DMH as compared to those treated with the carcinogen alone (data are not shown). At the end of experiment, intestinal tumors were found in the majority of rats (Table 7).

Macroscopically, these neoplasms were exophytic or endophytic. Several cases of ulcerative-infiltrative forms were observed as well. Microscopically, different types of malignant intestinal tumors were found, predominantly, tubular adenocarcinomas. Ca in situ, superficial carcinomas, mucinous and signet-ring carcinomas were also registered. All these types of carcinomas are typical for neoplasms induced by DMH (Pozharisski, 1990).

The data on the effect of diabenol on the development of colon tumors induced by DMH are presented in Tables 7 and 8. Total incidence of intestinal tumors was similar in both groups. However, the multiplicity (mean number of tumors per rat in a group) in animals treated with diabenol was decreased by 30% as compared with the parameter in rats exposed to DMH alone. Most expressed inhibiting effect of diabenol was revealed in ascending colon. The incidence of tumors of this site was decreased more than two times as compared to the control group, and the multiplicity of tumors was decreased by three times.

The claster analysis of distribution of animals with different numbers of colon tumors has shown that in control animals (DMH alone), the number of rats with six and more tumors per animal was 2.1 times higher than in the group treated with the carcinogen and diabenol (42.1% and 20%, correspondingly). Analysis of tumor size distribution has shown that in the descending colon of animals from the control group small tu-

Table 7
Effect of diabenol on 1,2-dimethylhydrazine (DMH)-induced colon tumorigenesis in rats

Parameter	DMH	DMH + diabenol
Number of rats	19	20
Number of tumor-bearing rats (%)	18 (94.7)	18 (90.0)
Colon (all parts)		
No. of tumor-bearing rats (%)	18 (94.7)	18 (90.0)
No. of tumors	104	76
No. of tumors per rat		
In group	5.47 ± 0.47	3.80 ± 0.47*
In tumor-bearing rats	5.77 ± 0.38	4.22 ± 0.45**
Mean tumor size (mm^2)	80.89 ± 33.86	51.93 ± 15.19
Ascending colon		
No. of tumor-bearing rats (%)	17 (89.5)	8 (40.0)***
No. of tumors	31	12
No. of tumors per rat		
In group	1.63 ± 0.13	0.60 ± 0.17***
In tumor-bearing rats	1.82 ± 0.80	1.50 ± 0.18
Mean tumor size (mm^2)	147.63 ± 69.11	84.39 ± 27.50
Descending colon		
No. of tumor-bearing rats (%)	17 (89.5)	18 (90.0)
No. of tumors	63	56
No. of tumors per rat		
In group	3.32 ± 0.44	2.80 ± 0.30
In tumor-bearing rats	3.71 ± 0.41	3.11 ± 0.26
Mean tumor size (mm^2)	57.44 ± 12.58	53.74 ± 14.17
Rectum		
No. of tumor-bearing rats (%)	9 (47.4)	6 (30.0)
No. of tumors	10	8
No. of tumors per rat		
In group	0.53 ± 0.13	0.40 ± 0.18
In tumor-bearing rats	1.10 ± 0.10	1.33 ± 0.25
Mean tumor size (mm^2)	37.6 ± 19.91	17.66 ± 3.90

* The difference from the controls is significant: $p < 0.05$.
** The difference from the controls is significant: $p < 0.01$.
*** The difference from the controls is significant: $p < 0.002$.

Table 8
Effect of diabenol on a distribution of colon tumors by growth pattern, differentiation rate and invasion depth (in percent to total number of colon tumors)

Parameter	DMH	DMH + diabenol
Growth pattern		
Exophytic	50	76.3
Endophytic	50	23.7
Differentiation rate		
High	14.7	47.4*
Moderate	61.6	34.2**
Low	23.5	18.4
Invasion depth		
Mucosa	17.6	34.2
Submucosa	35.3	34.2
Muscular layer	26.5	15.8
Serosa	20.6	15.8

* The significance from the control (DMH) is significant: $p < 0.001$ (Fischer's exact test).
** The significance from the control (DMH) is significant: $p < 0.05$.

mors (<51 mm^2) appeared less frequently in comparison with the group given diabenol (58.7% and 71.4%, correspondingly).

The results of morphological analysis (Table 8) have shown that the tumors with exophitic pattern of growth more frequently developed in diabenol-treated rats in comparison with the controls. Opposite situation was with endophytic colon tumors. Tumors in the group treated with diabenol more frequently were highly differentiated and less invasive as compared with the control group. Thus, these data show the inhibitory effect of diabenol on DMH-induced colon carcinogenesis.

4. Discussion

Our experiments have shown that the long-term treatment with the antidiabetic drug diabenol slowed down age-related disturbances in estrous function and increased life span of all and 10% most long-living female NMRI mice. The treatment with diabenol inhibited spontaneous tumor incidence and increased the mammary tumor latency in these mice. In short-living transgenic HER-2/neu mice, the drug also slowed down age-related changes in estrous function in HER-2/neu mice, slightly inhibited tumrigenesis but failed influence survival of these mice. In rats exposed to 1,2-dimethylhydrazine, the treatment with diabenol significantly inhibited colon carcinogenesis. These observations are in agreement with the data obtained with antidiabetic biguanides phenformin, buformin and metformin. It was shown in earlier studies that phenformin and buformin increase the life span and inhibits spontaneous carcinogenesis in female C3H/Sn mice (Dilman & Anisimov, 1980) and female outbred rats (Anisimov, 1980), inhibits colon carcinogenesis induced by DMH in rats (Anisimov, Pozharisski, & Dilman, 1980) as well as carcinogenesis induced by some other chemical carcinogens and ionizing radiation (Alexandrov et al., 1980; Anisimov, Belous, Vasilyeva, & Dilman, 1980; Anisimov, Ostroumova, & Dilman, 1980; Anisimov, Belous, & Prokudina, 1982; Bespalov & Alexandrov, 1985; Dilman, Berstein, Zabezhinski, Alexandrov, & Pliss, 1978; Vinnitski & Iakimenko, 1981). Treatment with metformin prolongs life span of rats (G. Roth, personal communication) and mice (McCarty, 2004) and inhibits pancreatic carcinogenesis in hamsters (Schneider et al., 2001).

Like the biguanides, diabenol slows down the age-related disturbanses in the estrus function of rodents. It is worthy of note that metformin improves menstrual regularity, leading to spontaneous ovulation, and enhanced the induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome (Awartani & Cheung, 2002; Nestler, Stovall, Akhther, Iorno, & Jakubowicz, 2002).

The use of phenformin in humans has been limited the last two decades because of an association with lactic acidosis. Metformin does not increase risk for lactic acidosis or increase lactate levels in type 2 diabetes (Kruse, 2004) but has some adverse effects, including renal insufficiency (Nisbet, Strurtevant, & Prins, 2004) and gastrointestinal side effects (Krentz, Ferner, & Balley, 1994).

Diabenol is not biguanide and belongs to derivatives of benzoimidazole. It seems that this drug could be free of adverse effects typical for biguanides. It was shown that diabenol decreases aggregation of thrombocytes and erhythrocytes as well as blood viscosity in diabetic animals (Spasov et al., 1997, 1999). This effect gives an additional advantage to use of diabenol as a geroprotector.

Several years ago, it was firstly suggested to use biguanide antidiabetics as mimetics of CR and a potential anti-aging treatment (Dilman, 1971, 1978, 1994). Although it is known that free radicals are produced

during metabolic reactions, it is largely unknown which factor(s), of physiological or pathophysiological significance, modulate their production in vivo. It has been suggested that hyperinsulinemia may increase free radicals, and therefore, promote aging, independent of glycemia (Facchini, Hua, Abbasi, & Reaven, 2001). Plasma levels of lipid hydroperoxides are higher, and antioxidant vitamins are lower in individuals who are resistant to insulin-stimulated glucose disposal but otherwise glucose tolerant, nonobese, and normotensive (Facchini et al., 2001). This finding indicates that enhanced oxidative stress is present before diabetes ensues, and therefore, cannot simply be explained by overt hyperglycemia. There is substantial evidence supporting the hypothesis that selective resistance to insulin-stimulated (muscle) glucose disposal and the consequential compensatory hyperinsulinemia trigger a variety of metabolic effects, likely resulting in accelerated oxidative stress and aging (Dilman, 1994; Facchini et al., 2000).

The anti-diabetics biguanides inhibit fatty acid oxidation, inhibit gluconeogenesis in the liver, increase the availability of insulin receptors, inhibit monoamine oxidase (Muntoni, 1999), increase sensitivity of hypothalamo–pituitary complex to negative feedback inhibition, reduce excretion of glucocorticoid metabolites and dehydroepiandrosterone-sulfate (Dilman, 1994). Recently it was shown that metformin decreases platelet superoxide anion production in diabetic patients (Gargiulo et al., 2002). Like antidiabetic biguanides, diabenol increases tissue glucose utilization in old obese rats and has antimutagen and antioxidant activities (Mezheritski et al., 1998; Spasov et al., 1997, 1999; Zinovieva et al., 2003).

It is worthy to note, that experiments in yeast and *Caenonhabditis elegans* show that the life extension by CR is not a mechanical output of low calories and consequence of a reduction in ROS or AGE formation, but a process that is highly regulated, triggering metabolic shift toward respiration that activates the regulator SIR2 (Koubova & Guarente, 2003). In yeast and worms, life span is extended by extracopies of *SIR2/Sir*-2.1 gene (Tissenbaum & Guarente, 2001), by *SIR2* orthologue, *Sirt1* (sirtuin 1) (Picard et al., 2004), or by small molecule sirtuin-1 agonists, e.g. resveratrol (Howitz et al., 2003). In mammals, it is suggested that SIRT1 is a key regulator of cell defences and survival in response to stress (Brunet et al., 2004; Motta et al., 2004). Recently, it was shown that the expression of mammalian Sir2 (SIRT1) is induced in CR rats as well as in human cells that were treated with serum from these animals (Cohen et al., 2004). Long-lived mutant mice and CR rodents are protected from cancer despite attenuating apoptosis possibly because their cells possess increased defences and repair mechanism and they retain the ability to undergo apoptosis if the damage is beyond repair (Cohen et al., 2004). It was observed that phenformin inhibits proliferation and induced enhanced and transient expression of the cell cycle inhibitor p21 and apoptosis in human tumor cells lines (Caraci et al., 2003). The possibility that diabenol stimulate an active defence response in the animal can not to be excluded and needs an experimental testing. Thus, the results of our experiments together with the data and findings discussed above provide evidence that diabenol is promising geroprotector and anticarcinogen.

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