BIOGERONTOLOGY

Effect of Neuronol on Lifespan and Development of Spontaneous Tumors in SAMP-1 Mice with Genetically Accelerated Aging

I. G. Popovich, M. A. Zabezhinskii, V. N. Anisimov, A. L. Kovalenko*, A. Yu. Petrov*, A. V. Semenchenko, and A. I. Yashin****

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Treatment of female SAMP-1 mice with Neuronol (drug containing succinic acid) given with drinking water starting from the age of 2 months during the whole life prolonged the lifespan and markedly reduced mortality of animals aged 1.5-2 years. Neuronol inhibited the development of spontaneous tumors, primarily lymphomas, and significantly prolonged lifespan in mice with tumors. Long-term treatment with Neuronol had no pathological side effects. Our experiments demonstrated geroprotective and anticarcinogenic activity of Neuronol and safety of its long-term use.

Key Words: *Neuronol; succinic acid; geroprotective effect; anticarcinogenic effect; SAMP-1 mice*

The search for agents preventing untimely aging is a pressing problem of gerontology [6]. Age-associated metabolic rearrangements consist in modification of mitochondrial energetics (inhibition of succinic acid oxidation), which is proven by the data on decreased SDH activity in tissues during aging [5]. Factors activating the system of succinic acid formation and utilization in the body can effectively improve its functional potentialities. Treatment with succinate-containing drugs can prolong the lifespan of laboratory animals [7]. We studied the effect of Neuronol (drug containing succinic acid) on the processes of aging and spontaneous tumor development in mice with genetically accelerated aging. SAMP-1 (senescence accelerated mouse-prone-1) strain was derived by selection of AKR/J mice [9,11]; it is characterized by increased production of reactive oxygen species (ROS) and high level of 8-hydroxyguanine in organs [4,11]. Presumably, mitochondrial electron transporting system is impaired in SAMP-1 mice [9].

MATERIALS AND METHODS

Experiments were carried out on 63 female SAMP-1 mice, belonging to generations 103 and 104 of mice given by Department of Embryology, Biological Faculty of Moscow State University, and maintained at N. N. Petrov Institute of Oncology. The animals were kept in plastic cages, 10 per cage, at 21-23°C at common day/night regimen (12/12 h) on standard granulated fodder with free access to water. Neuronol (Polisan) contained succinic acid (36.5%), piracetam (25.5%), riboxine (25.5%), nicotinamide (7.3%), riboflavine mononucleotide (2.6%), and pyridoxine hydrochloride (2.6%). The drug is a bright yellow powder with ascorbic acid odor, easily dissolved in water. At the age

Department of Carcinogenesis and Oncogerontology, N. N. Petrov Institute of Oncology, Ministry of Health of Russian Federation; *Polisan Firm, St. Petersburg; **Max Planck Institute of Demographic Studies of Society, Restock. ***Address for correspondence:*** aging@mail.ru. Anisimov V. N.

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of 2 months the animals were randomly divided into 2 groups: 1) (n=32) intact controls and 2) (n=31) treatment with Neuronol dissolved in drinking water (500 mg/l) 5 times a week throughout the whole life. All animals were monthly weighed, daily consumption of fodder and water was evaluated every 3 months. Throughout the experiment vaginal smears were examined under microscope for evaluating estrous function for 2 weeks. The duration of the cycle and its phases, the ratio of the number of cycles with different duration, ratio of the number of individual phases of the cycles and the number of regular and irregular cycles were recorded. Rectal temperature was measured every 3 months using a TPEM-1 electric thermometer. Each death was recorded. After death the animals were autopsied. All tumors and viscera were fixed in 10% neutral formalin and after standard histological processing the sections were stained with hematoxylin and eosin. The tumors were classified in accordance with the criteria of the International Cancer Research Agency [10]. The results of the experiments were statistically processed using Student's t test and accurate Fisher's method [2]. Survival was analyzed using Cox' method [7]. Mathematical Homperz model was used to described the survival function:

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

where α and β parameters characterized the population rate of aging and initial mortality, respectively. The time of mortality doubling was estimated as $\ln(2)/\alpha$. The parameters of the model were estimated on the base of the maximum likelihood method, using Gauss statistical system [8]. 95% confidence intervals for aging parameters were estimated using Cox' method [7].

RESULTS

The animals well tolerated Neuronol treatment. No signs of drug toxicity were detected. The drug did not affect the age-specific dynamics of body weight and food consumption (varied from 3.4 to 4.1 g/mouse/day). Water consumption decreased starting from the age of 6 months in comparison with the control, these differences were statistically significant at the age of 6, 12, and 18 months (Table 1). Estrous function is a well-known biological marker of aging. We found that the mean duration of the estrous cycle increased with age in both groups, which is characteristic of aging animals. No significant differences between the groups were observed at the age of 3-15 months. Only at 17 months the mean duration of the estrous cycle in experimental mice was shorter than in the control (5.90±0.34 and 6.80±0.55 days, respectively), though the differences were insignificant. The relative incidence of irregular cycles increased starting from the age of 12 months in both groups, which also indicates age-associated disorders in hormonal balance. No statistically significant differences in this parameter were detected between the two groups. Hence, Neuronol had no adverse effect on the estrous function of mice and even prevented age-specific changes in 17-month-old animals. Neuronol had no definite effect on body temperature.

The mean lifespan of mice significantly increased under the effect of Neuronol (by 12.2%, p<0.05; Table 2) and the survival curve acquired a rectangular pattern (Fig. 1, *a*), which also indicated prolongation of mouse lifespan under the effect of the drug. Log rank analysis showed the significance of differences between the survival curves (p<0.01). Hence, Neuronol possesses a pronounced geroprotective effect.

The first tumors in mice treated with Neuronol were detected 150 days later than in the control group (Table 2; Fig. 1, *b*). At older age tumors of different location developed later than in the control. The mean lifespan of animals with tumors treated with Neuronol was by 70 days longer than in the control group (p<0.05). Autopsy showed enlarged liver, spleen, thymus, peripheral lymph nodes (submaxillary, axillary, inguinal) and lymph nodes in the thoracic cavity (peribronchial, periaortal) and abdominal cavity (mainly mesenteric). Microscopic examination showed tissue infiltration with tumor cells in the lymph nodes, spleen, liver, kidneys, and lungs. These tumors were classified as malignant lymphomas. Another type of tumors was malignant fibrous histiocytomas, usually developing under the skin of the back or hip and rapidly growing; these tumors had metastases. The incidence of tumors

TABLE 1. Water Consumption in Mice Receiving and Not Receiving Neuronal (ml/mouse/day)

Group	Age, months				
	3	6	9	12	18
Control	5.10±0.02	6.60±0.58	6.30±0.15	9.10±0.31	7.80±0.38
Neuronal	6.00±0.58	5.10±0.24**	6.00±0.56	5.40±0.31*	6.40±0.56***

Note. *p<0.01, **p<0.02, ***p<0.05 compared to the control.

TABLE 2. Effect of Neuronal on Mean Lifespan (MLS) and Incidence of Spontaneous Tumors in SAMP-1 Mice

Parameters	Control (*n*=32)	Neuronal (*n*=31)
MLS, days	570.0±22.4	640.0±20.6**
MLS of the last 10% mice, days	674.0±4.6	776.0±14.2*
Maximum lifespan, days	819	815
Rate of population aging α, 1×10^{-3}, day^{-1}	9.3 (9.2; 9.9)$^{+}$	11.7 (11.3; 12.1)$^{+}$**
Time of mortality doubling, days	74.6 (70.1; 75.6)$^{+}$	59.0 (57.1; 61.6)$^{+}$**
Day of detection of the 1st tumor	280	430
MLS of mice with tumors	586.0±25.4	656.0±20.8**
Number of mice with tumors	28.0 (87.5%)	23.0 (74.2%)
Tumor types		
generalized malignant lymphomas	27.0 (84.4%)	23.0 (74.2%)
malignant fibrous histiocytomas of the subcutaneous fat	3.0 (9.4%)	1.0 (3.2%)
ovarian cysts	18.0 (56.3%)	17.0 (54.8%)

Note. *p<0.01, **p<0.05 compared to the control; $^{+}$95% confidence interval is shown in parentheses.

was observed in the group receiving Neuronol tended to decrease (p>0.05).

Of other pathologies we observed hemorrhagic ovarian cysts, which were about equally incident and appeared with the same latency in both groups of animals. The presence of these cysts could be an indicator of hormonal imbalance in SAMP-1 mice, presumably favoring aging and shortening of the lifespan.

Experiments demonstrated pronounced geroprotective and anticarcinogenic effects of Neuronol. These data are in line with reports on similar effect of succinic acid [1,3]. The effects of both agents were most pronounced in long-living (more than 500 days) mice. Extrapolating these data on humans, we expect that the most pronounced effects of succinic acid preparations will be observed in subjects of medium and elderly age. The use of succinic acid in old rats was associated with improvement of many metabolic parameters and increase in their lifespan [3].

The inhibitory effect of succinic acid on tumor development can be associated with its normalizing effect on energy processes in mitochondria. The normalizing effect of succinate on energy supply of the neuroendocrine system seems to be essential, especially the capacity of succinic acid to reduce the threshold sensitivity of the hypothalamo-pituitary system to homeostatic signals, which can underlie its geroprotective effect. The absence of side effects during long-term treatment with Neuronol in experimental animals indicates its safety and prompts clinical studies.

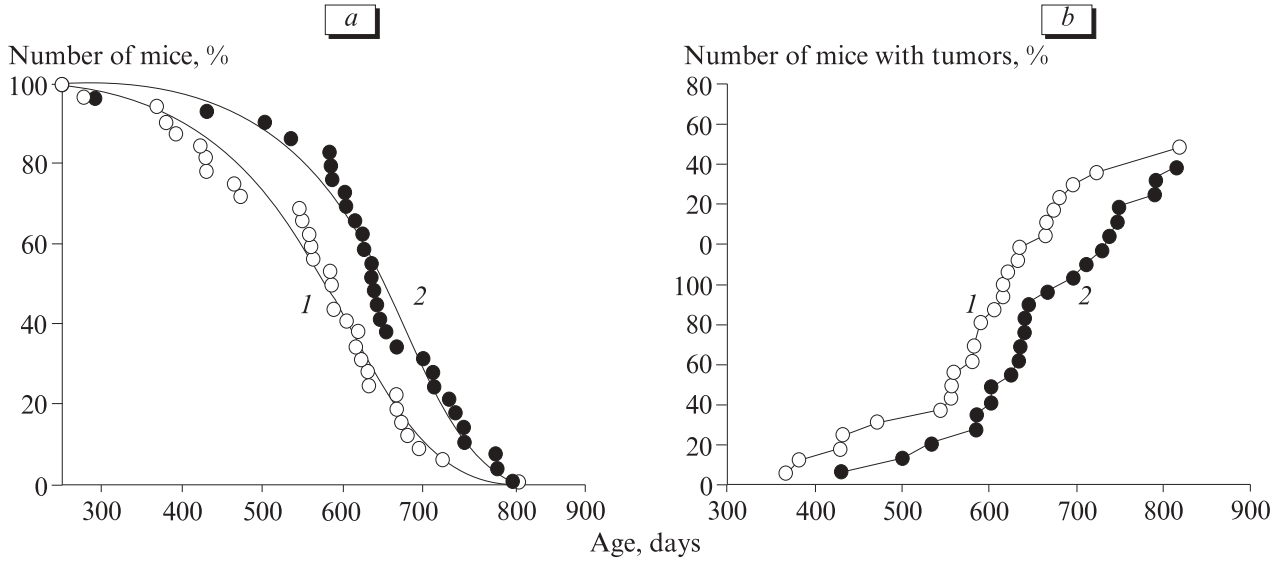

Fig. 1. Effect of Neuronol on total survival of SAMP-1 mice (*a*) and their mortality from lymphomas (*b*). *1*) control; *2*) Neuronol.

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