BIOGERONTOLOGY

Artificial Aging of Mice

V. A. Zuev, G. G. Avtandilov*, N. G. Ignatova, and S. I. Bykovskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 325-327, September, 2003
Original article submitted April 21, 2003

Clinical signs of aging verified by morphometrical analysis of brain tissue were observed in young mice 4 months after administration of brain extract from old mice (5 intraperitoneal injections).

Key Words: *cytoproliferative factor; glia; morphometry*

A factor sharply stimulating proliferation of glial cells was detected in brain extracts from aging mice [2]. We hypothesized that this factor disturbing neuronal metabolism plays a key role in the processes of brain aging and death [1,3]. However it remained unclear whether the detected factor is the cause of enhanced glial proliferation or a result of this process and hence, the result of aging.

In order to clear out whether glial proliferation factor is the cause of this phenomenon, we induced artificial aging in young mice.

MATERIALS AND METHODS

Brain extracts from 2-year-old C57BL/6 mice were used. The brain was removed, crushed in a ceramic mortar with fused corundum, 1 ml 0.85% sodium chloride was added, and after pipetting the suspension was centrifuged for 15 min at 2000 rpm. The supernatant was diluted 10-fold, heated for 30 min, recentrifuged for 15 min at 2000 rpm, and filtered through Millex-HA filters (0.45 μ, Millipore SA).

The filtrate was injected daily for 5 days to 1.5-month-old mice of the same strain supraorbitally (0.1 ml) and intraperitoneally (0.2 ml). Controls (mice of the same strain) were injected with 0.1 or 0.2 ml of brain extract from 1.5-month-old mice according to the same protocol.

For morphometrical study, the brain of narcotized mice was dissected with a razor along the frontal plane in the middle of the hemispheres. The fragments were fixed in 10% formalin, dehydrated in ascending alco-

TABLE 1. Cytoproliferative Activity in Sera of Young Mice Injected with Brain Extracts from Young and Old Mice

Route of injection, group	Age, months	
	3	5.5
Supraorbital		
control	1.05	1.1
	1.07	1.1
experiment	2.0	1.4
	1.8	1.4
	1.6	1.3
	1.7	1.3
	1.5	1.6
Intraperitoneal		
control	0.9	1.1
	1.0	1.1
experiment	1.3	1.5
	1.3	1.5
	1.2	1.4
	1.3	1.5
	1.2	1.5

N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences; *Russian Medical Academy for Advanced Training, Moscow. ***Address for correspondence:*** zuev@home.domonet.ru. Zuev B. A.

0007-4888/03/1363-0286$25.00

TABLE 2. Morphometrical Parameters of Histological Sections of Standard Areas in Mouse Cerebral Cortex (*M*±*m*)

Group	Number of objects	Area of nuclei in square pixels	Integral brightness, arb. units	Volume rate of neurons, %
Control	62	440±12	75,136±1653	12
Experiment (after injections of brain extract from old mice)	64	393±15	65,601±3371	7

hols, and embedded in paraffin. Histological sections (5 μ) were stained with hematoxylin and eosin, with picrofuscin by the method of Van Gieson, by the method of Feulgen, or impregnated with silver. After routine pathohistological examination, stereological analysis was carried out using a 100-point Avtandilov ocular grid, and the images were analyzed using an IMAGER-CG computer complex equipped with Avtan-San software. The number of elements, area of neuronal nuclei, integral brightness (reflecting the amount of the substance), and cell ploidy were evaluated.

The data were presented as the means (simple and suspended), dispersion of the values, and error of the sampling.

RESULTS

The blood was collected from the supraorbital sinus individually from all mice 1.5 months after the start of the experiment (*i.e.* at the age of 3 months). The serum separated from the clot was diluted 10-fold and its cytoproliferative activity was measured using L_{929} cell culture (by the ratio of cell grown in the presence and absence of serum; Table 1). No cytoproliferative activity was detected in the sera of control mice at this term (Table 1). However this activity (very low) was present in the sera from both groups of experimental animals, being somewhat higher in animals receiving supraorbital injections. This probably was due to injection of the test material directly into the blood. Body weight remained unchanged (~25 g for each mouse).

Four months after the start of the experiment (at the age of 5.5 months) the blood was collected again. The sera from control mice exhibited no cytoproliferative activity, while in experimental animals it increased, especially in animals receiving intraperitoneal injections (Table 1). This can be explained by peculiarities of intraperitoneal administration of the brain extract: the material was absorbed more slowly.

All animals were inert and demonstrated slack reaction to food, the fur became lusterless and thick, in one mouse gray hairs appear. Body weight was by 1.0-1.5 g below the control. At the same time control mice were active, with active reaction to food, and with shiny fur.

In order to confirm clinical observations and serological findings, morphometrical analysis of the brain was carried out. The mice from the control and experimental (gray mouse) groups were sacrificed and morphometrical study of histological sections of the brain cortex was carried out (Table 2). In a control mouse the volume rate of neurons was 12% *vs.* 7% in the mouse receiving 5 intraperitoneal injections of the cytoproliferative factor: the number of neurons in the cerebral cortex drastically decreased (Table 2).

The decrease in the number of neurons in the brain of aging mammals, including humans, is a cardinal sign of their natural aging [4]. These changes were first reported for mice [5].

Previous studies demonstrated accumulation of the cytoproliferative factor in aging mice starting from the age of 10 months [1]. However, no clinical signs of aging were detected at this age [1]. The decrease of the volume rate of cerebrocortical neurons in 1.5-year-old mice did not exceed 10% of this value in 1.5-month-old animals.

Hence, clinical signs of artificial aging of young mice in our experiments are associated with a characteristic drop in the number of cerebrocortical neurons. This indicates that accumulation of cytoproliferative factor in aging mice [2,3] is the cause, but not a result of brain aging and death.

REFERENCES

1. V. A. Zuev, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 11, 46-49 (2001).
2. V. A. Zuev, I. V. Viktorov, N. P. Borodina, *et al.*, *Byull. Eksp. Biol. Med.*, **129**, No. 3, 317-320 (2000).
3. V. A. Zuev, I. V. Viktorov, N. G. Ignatova, *et al.*, *Neurodegenerative Diseases and Aging*, Eds. by I. A. Zavalishin, N. N. Yakhno, S. I. Gavrilova [in Russian], Moscow (2001), pp. 261-276.
4. C. L. Masters, G. Multhaup, G. Simms, *et al.*, *EMBO J.*, **4**, 2757-2763 (1985).
5. H. Sobel, *Lancet*, **2**, No. 7684, 1191-1192 (1970).