BIOGERONTOLOGY

Carnosine Modifies the Incidence of Genetically Abnormal Sex Cells in the Testes of Senescence-Accelerated Mice

S. T. Zakhidov, A. V. Gopko, M. L. Semenova, Ya. Yu. Mikhaleva, A. A. Makarov, and A. Yu. Kulibin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 89-92, July, 2002
Original article submitted March 26, 2002

The quantitative micronucleus test showed that the natural dipeptide carnosine increases the count of aberrant spermatogonia and round spermatids in the testes in SAMR1 mice resistant to accelerated aging by 64 and 85%, respectively, compared to the control. However, this agent did not modify the incidence of chromosome mutations in spermatogenic cells in SAMP1 mice predisposed to accelerated aging.

Key Words: *carnosine; SAMP1 mice; SAMR1 mice; spermatogenic cells; micronuclei*

Carnosine is a natural histidine-containing dipeptide that normalizes and stimulates functional activity of the antioxidant system. This compound possesses immunostimulating and geroprotective properties, prolongs cell lifespan *in vitro*, protects genetic structures from genotoxic factors, and kills transformed cells [1-3,11].

Rapidly aging mice (SAM, senescence-accelerated mouse) were bred in Japan by selective inbreeding of animals produced after casual mating of AKR/J mice with hereditary leukemia and unknown mice [14, 15]. Inbred mice were divided into groups of animals predisposed and resistant to accelerated aging (SAMP and SAMR, respectively). Despite the same phylogenetic age of SAMP1 and SAMR1 mice, the maximum life span of these animals is 15 and 24 months, respectively (average 9.9 and 18.3 months, respectively). In many experiments SAMR1 mice serve as the "natural background" (negative control).

Our previous studies showed that in SAMP1 and SAMR1 mice the incidence of male sex cells with chromosomal aberrations markedly surpasses the limits of spontaneous mutability for spermatogenesis in normally aging animals [6,7].

Here we studied whether carnosine can decrease the incidence of chromosomal mutations in cells of the spermatogenic epithelium in rapidly aging mice.

MATERIALS AND METHODS

Experiments were performed on male SAMP1 (n=16) and SAMR1 mice (n=28). The animals were kept in a vivarium under standard conditions and had free access to food and water. Starting from the 2nd month of life some males (experimental group) received freshly prepared aqueous solution of carnosine in a daily dose of 100 mg/kg [4]. One-year-old mice were killed by cervical dislocation.

Samples of spermatogenic cells were prepared, fixed, and stained as described elsewhere [5].

The incidence of genetic abnormalities in spermatogonia and round spermatids was estimated by calculating micronuclei resulting from fragmentation and/or loss of chromosomes during mitotic and meiotic divisions. The quantitative micronucleus test is widely used in mutational genetics and genetic toxicology.

Department of Embryology, Biological Faculty, Institute of Mechanics, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** STZ-su@mail.ru. Zakhidov S. T.

0007-4888/02/1341-078$27.00

This technically simple and productive method provides reliable information on the arrangement of chromosomal materials in somatic and sex cells and its deviations from normal under the influence of various factors. We calculated not less then 500 spermatogonia and 1000 round spermatids from each animal. The count of aberrant cells was expressed in $^{o}/_{oo}$.

The results were analyzed by SPSS software. The significance of differences was estimated by Wilcoxon test.

RESULTS

Long-term treatment with carnosine insignificantly changed the average incidence of spermatogonia and round spermatids with micronuclei in SAMP1 mice. Carnosine increased the count of cells with chromosome mutations by 20% compared to the control (Table 1). By contrast, in SAMR1 mice receiving carnosine the incidence of genetically abnormal spermatogonia and round spermatids considerably surpassed the control (by 64 and 85%, respectively). These results show that carnosine possesses no antimutagenic activity, but produced an opposite effect.

The incidence of spermatogonia with micronuclei was surprisingly high in some control and experimental SAMP1 mice. In statistics these values are known as outliers. Similar results were obtained after cytogenetic analysis of round spermatids from control SAMR1 mice (Fig. 1). Comparison of arithmetic means by Wilcoxon test after exclusion of outliers produced the same results.

One-way analysis of variance (Leven test) showed that in SAMR1 mice receiving carnosine variations in the incidence of round spermatids with micronuclei were higher than in the control. Therefore, carnosine increased the degree of genetic chaos in the population of round spermatids in SAMR1 mice. In other experiments we revealed no significant differences in the homogeneity of variations (Leven test).

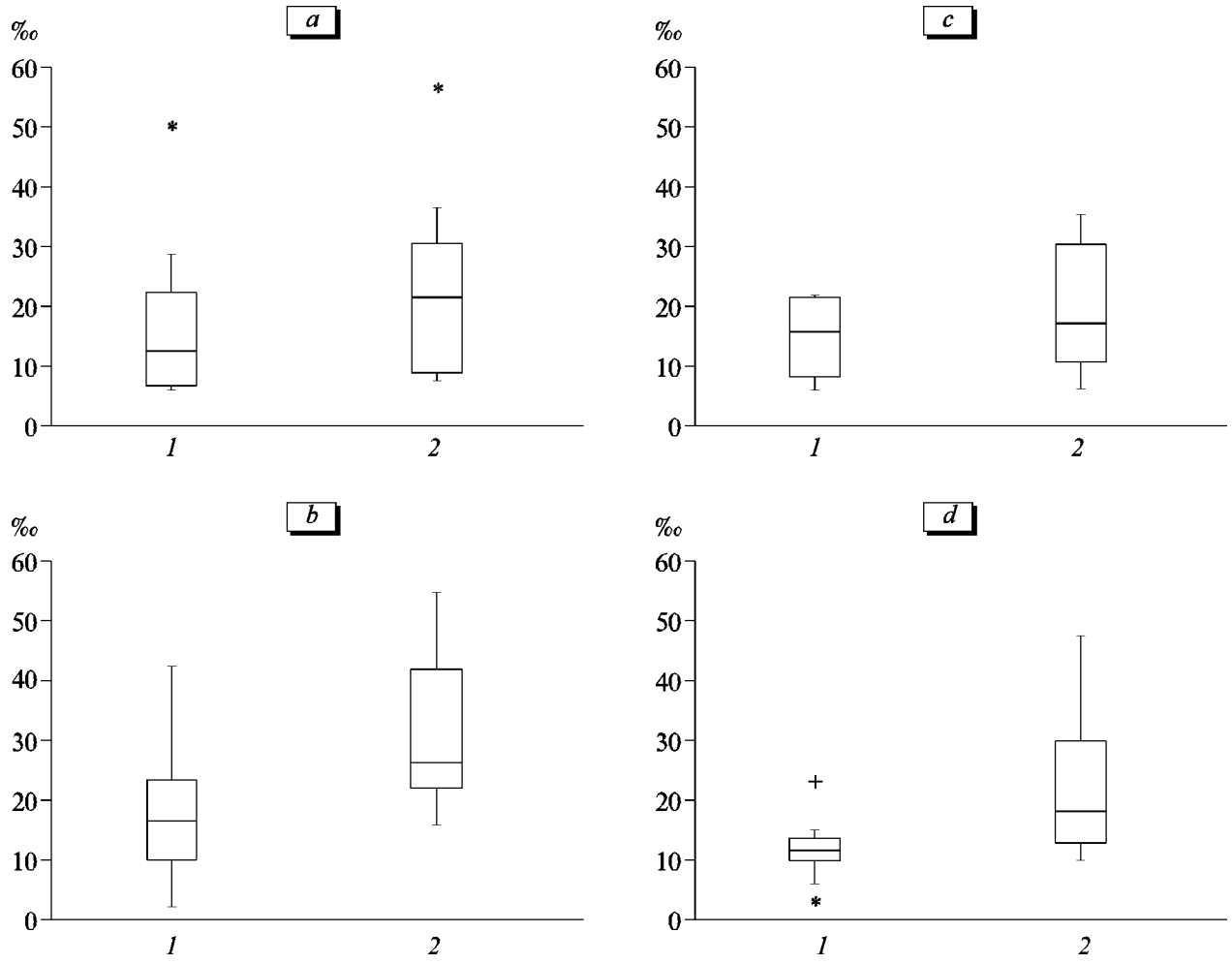

Fig. 1. Incidence of aberrant spermatogonia (*a*, *b*) and round spermatids (*c*, *d*) in SAMP1 (*a*, *c*) and SAMR1 mice (*b*, *d*): control (*1*) and carnosine (*2*). Box: magnitude between the first and third quartiles. "Whiskers": amplitude of distribution. Horizontal line inside the box: median (50% quartile). Outliers: *moderate, +pronounced.

TABLE 1. Incidence of Spermatogenic Cells with Micronuclei in Testes of Control and Experimental SAMP1 and SAMR1 Mice ($^{o}/_{oo}$, M±m)

Cells	SAMP1		SAMR1	
	control (*n*=7)	experiment (*n*=9)	control (*n*=16)	experiment (*n*=12)
Spermatogonia	18.8±5.9	23.1±5.2	19.1±3.2	31.4±3.6**
Round spermatids	15.6±2.4	19.9±3.6	11.9±1.3	22.0±3.2*

Note. *p=0.002 and **p=0.008 compared to the control.

It remains unclear whether carnosine directly modifies the genetic apparatus. Our results suggest that higher incidence of micronuclei in the testes of SAMR1 mice is associated with increased genetic instability due to carnosine-induced changes in enzyme activities. These changes make impossible the effective and strict control over progressive genetic instability (spontaneous mutability). It cannot be excluded that accumulation of cells with micronuclei in the testes of SAMR1 mice receiving carnosine is related to disturbances in the mechanism of cell death (selection). This mechanism involves interaction and balance between specific controlling proteins (p53, Bcl-x, and Bax) that act during spermatogonium division and prophase I of meiosis [10,12,13]. Disturbances in the mechanism of cell elimination modulate proliferative activity and promote survival of genetically unstable cells that should be killed.

It can be hypothesized that in 12-15-month-old SAMP1 mice long-term treatment with carnosine did not disturb cell selection.

Our results show that carnosine produced an unusual modifying effect on developing spermatogenic cells in SAMP1 and SAMR1 mice. It should be emphasized that under these experimental conditions carnosine affects the systems that are formed, develop, and function against the background of high genetic instability and activation of compensatory processes. The number of aberrations in these systems increases many times and chaotically over a short period (compared to normal). In systems characterized by marked deviations from normal the behavior of innovating molecules can be unstable and unpredictable.

Further ontogenetic studies with younger animals should provide complete information on the effect of carnosine on genetic structures and development of male sex cells in senescence-accelerated mice.

I. A. Rapoport [8,9] wrote that cells cannot be immortal even under most favorable conditions, because normal catalytic process is affected by the environment and inorganic enzyme poisons. These compounds hinder biocatalytic processes. All attempts to reduction of the influence of phenotypic environmental factors by affecting the genotype are now hopeless. Under these conditions carnosine acting as a potent modifying agent in optimal concentration and/or in combination with other factors can decrease mutational and biological entropy and decelerate aging.

This work was supported by the Russian Foundation for Basic Research (grant No. 00-04-48155a).

REFERENCES

1. *Biochemistry* [in Russian], Vol. 65 (2000).
2. A. A. Boldyrev, *Carnosine* [in Russian], Moscow (1998).
3. A. A. Boldyrev, *Biokhimiya*, **65**, 884-890 (2000).
4. S. Gallant, M. L. Semenova, and M. Yuneva, *Ibid*., 1018-1021 (2000).
5. S. T. Zakhidov, L. P. Paranyushkina, Kh. A. Kh. Makhran, *et al*., *Izv. Ros. Akad. Nauk. Ser. Biol*., No. 3, 353-362 (1994).
6. S. T. Zakhidov, M. L. Semenova, O. F. Gordeeva, and A. A. Belyaeva, *Dokl. Ros. Akad. Nauk*, **365**, 273-275 (1999).
7. S. T. Zakhidov, O. F. Gordeeva, and T. L. Marshak, *Izv. Ros. Akad. Nauk. Ser. Biol.*, No. 1, 23-30 (2001).
8. I. A. Rapoport, *Chemical Mutagenesis and Selection* [in Russian], Moscow (1971).
9. I. A. Rapoport, *Chemical Mutagenesis in Selection* [in Russian], Moscow (1987).
10. K. Boekelheide, J. Lee, E. B. Shipp, *et al*., *Toxicol. Lett*., **102**, 25-34 (1998).
11. A. R. Hipkiss, *Int. J. Biochem*., **30**, 863-868 (1998).
12. C. G. Print, K. L. Loveland, L. Gibson, *et al*., *Proc. Natl. Acad. Sci. USA*, **95**, 12,421-12,431 (1998).
13. D. Schwartz, N. Goldfinger, Z. Kam, and V. H. Rotter, *Cell Growth Differ*., **10**, 665-675 (1999).
14. T. Takeda, M. Hosokawa, S. Takeshita, *et al*., *Mech. Aging Dev*., **17**, 183-194 (1981).
15. T. Takeda, M. Hosokawa, and K. Huguhi, *Exp. Gerontol*., **32**, 105-109 (1997).