Effect of Zosterin on Protein-Synthesizing Activity of Hepatocytes

M. N. Sgrebneva, V. I. Tsygankov*, A. P. Anisimov, and E. I. Khasina**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 10, pp. 426-428, October, 2005
Original article submitted January 27, 2005

The effect of zosterin (pectin polysaccharide) on the protein-synthesizing function of mouse liver cells was studied after its intragastric administration of 1% gel zosterin. The drug modified the morphology and function of the nucleolar apparatus by changing the number and summary area of the nucleoli, content of Ag proteins, nucleolar/nuclear ratio, and increased protein content in hepatocyte cytoplasm.

Key Words: *protein; hepatocytes; zosterin; nucleolar system*

Drugs based on polysaccharides of plant and animal origin are now widely used in clinical and preventive medicine. Physiological and metabolic aspects of the effects of polysaccharides on the liver in some diseases are most extensively studied. It was previously shown that zosterin optimized liver metabolism and improved nonspecific resistance of the body in hypoxia, exercise, and toxic hepatitis [7,8]. When injected to intact animals, the drug modified the endocrine metabolic status of the body and improved its adaptation potential [8]. Nonspecific mechanisms of organ and body resistance are realized primarily through energy and plastic metabolism. Zosterin is a hepatotropic drug, but the mechanism of its effect remains not quite clear.

We studied the effects of zosterin on protein-synthesizing function of liver parenchymal cells in intact animals.

MATERIALS AND METHODS

The study was carried out on adult outbred male mice (20-25 g). Care and all manipulations with the animals were carried out in accordance with the European Convention for Protection of Experimental Animals (1986, EEC).

Institute of Biology of the Sea, Far Eastern Division of Russian Academy of Sciences, Vladivostok; *Far Eastern State Medical University, Khabarovsk; **Far Eastern State University, Vladivostok

Experimental animals (n=7) daily received a single intragastric dose of zosterin (100 mg/kg, 1% gel) for 14 days; intact animals (n=7) received an equivalent volume of normal saline. Zosterin isolated from *Zostera marina L.* contains 74.8% D-galacturonic acid; the degree of its esterification is 5.7%, characteristic viscosity 340 ml/g galacturonane. The animals were decapitated under light ether narcosis 24 h after the last dose of the drug.

Liver fragments were fixed in cold ethanol-acetic acid (3:1) mixture and treated as described previously [1]. For identification of specific nucleolar proteins the preparations were stained with 50% colloid silver nitrate as described previously [5]. The area of hepatocyte nuclei and number and area of nucleoli were evaluated. The nucleolus/nucleus ratio (ratio of total nucleolar area to the area of the same nucleus) was estimated for evaluating the percentage of nucleoli per unit of nuclear area. On average, 100 nuclei per liver specimen were measured.

Smears of isolated liver cells were prepared as described previously [4] and fixed in absolute methanol for 15 min. The preparations were stained with 0.03% Amido Black 10 B solution for 1 h [3]. Protein content in hepatocyte cytoplasm was estimated as the product of optical density and cytoplasm area.

Nuclear ploidy was evaluated by their area, because these parameters of liver cells are in good correlation [2]. The ploidy of binuclear hepatocytes was

0007-4888/05/1404-0425

evaluated as the sum of both nuclei. On average, 70 hepatocytes in the liver of each animal were examined. The study was carried out on a MEKOS-C1 image analyzer. The significance of differences between the groups was evaluated by Student's *t* test using Statistica software.

RESULTS

Zosterin induced a 33% increase in the number of nucleoli in hepatocyte nuclei; the mean area of some nucleoli decreased by 17% (Fig 1).

The summary area of the nucleoli and content of Ag proteins in them increased by 5 and 22%, respectively (to 82.1±6.3 μ^2 and 47.6±6.4 arb. units). In intact group these parameters were 78.2±3.3 μ^2 and 39.0±4.7 arb. units, respectively. The nucleolus/nucleus ratio in experimental group increased 1.5-fold compared to the intact group (Fig. 2).

Zosterin treatment resulted in redistribution of liver cells by the ploidy classes (Table 1) *vs.* the normal level of 6.3c. Zosterin treatment promoted a 24% decrease in the percentage of diploid cells and 9 and 41% decrease in the counts of high-ploid (8c and 16c) cells, respectively, while the percentage of tetraploid cells increased by 3%. The mean ploidy decreased to 5.6c. Changes in the levels of cells of different classes were insignificant.

Zosterin treatment increased protein content in hepatocyte cytoplasm (Fig. 3), which reflects their significant functional strain. Protein content in diploid cells increased by 76%, in tetraploid cells by 29%, in octaploid cells by 30%, and in 16c cells by 18%.

The increase in the percentage of tetraploid hepatocytes in parallel with decrease in the percentage of cell of other ploidy classes suggested that zosterin can stimulate proliferative activity of cells. Pectins, particularly low-esterified, bind DNA in tissues of the liver, kidneys, and other organs and modify proliferation intensity [10,11]. Diploid hepatocytes proved to be the most sensitive to zosterin. They started the mitotic cycle which led to the appearance of cells of the next ploidy level (4c) and exhibited the most pronounced changes in the protein content in the cytoplasm. In addition, zosterin led to disaggregation of fused nucleolar organizers in hepatocyte nuclei, as the number of nucleoli increased, while their mean area decreased. This could be due to increased proliferative activity of hepatocytes and activation of latent nucleolus-forming chromosome regions (their transition into a more active functional state) [9]. Usually the increase in the number of nucleoli and nucleolus/nucleus ratio reflects increased intensity of protein synthesis in cells during normal development and hyperfunction of organs [12,13]. Our experiments demonstrated zosterin-induced increase in protein production

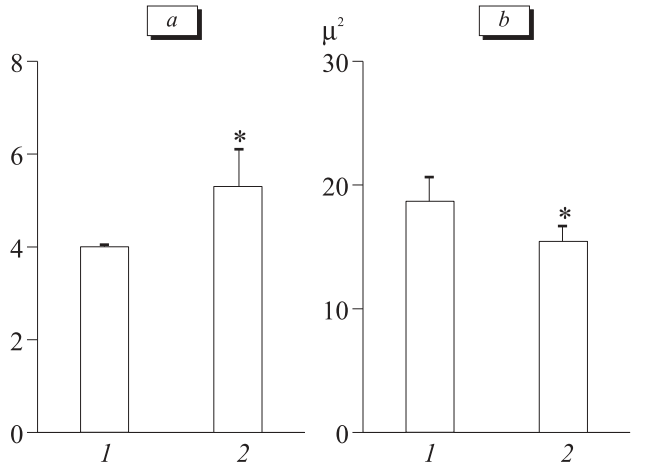

Fig. 1. Effect of zosterin on the number (*a*) and mean area (*b*) of nucleoli in mouse hepatocyte nuclei. Here and in Fig. 2: *1*) intact mice; *2*) mice treated with zosterin. *$p<0.05$ compared to intact animals.

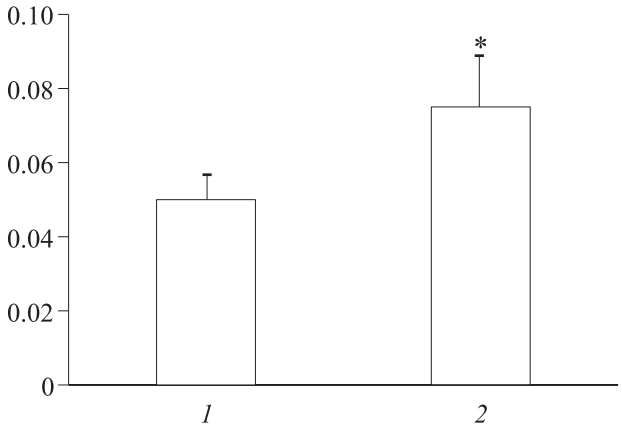

Fig. 2. Effect of zosterin on nucleolus/nucleus ratio in mouse hepatocytes.

TABLE 1. Ploidy Redistribution of Mouse Hepatocyte Nuclei under the Effect of Zosterin (*M*±*m*, %)

Group	Ploidy class			
	2c	4c	8c	16c
Intact	6.3±0.5	55.7±1.6	38.5±1.7	5.1±0.7
Experimental	4.8±1.1	57.5±2.5	35.1±3.1	3.0±0.6

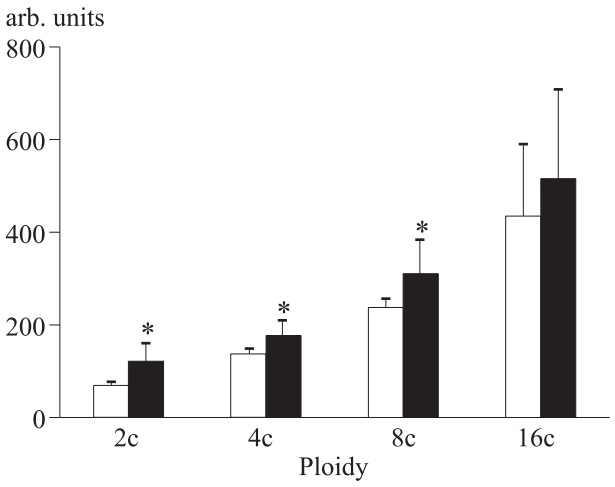

Fig. 3. Effect of zosterin on protein content in hepatocyte cytoplasm. Light bars: intact animals; dark bars: animals treated by zosterin. *$p<0.05$ compared to intact animals.

by mouse hepatocytes. The increase in protein synthesis leads to accumulation of plastic material, hyperproduction of enzymes, and hence, to their activation. This intensification of metabolic processes improves hepatocyte resistance and promotes optimal development of cellular and intracellular mechanisms of tissue regeneration [6].

The authors are grateful to Prof. S. S. Timoshin, Head of Research Laboratory, Far-Eastern State Medical University, for assistance.

REFERENCES

1. A. P. Anisimov, *Tsitologiya*, **34**, Nos. 11-12, 110-112 (1992).
2. V. Ya. Brodskii and I. V. Uryvaeva, *Cell Polyploidy. Proliferation and Differentiation* [in Russian], Moscow (1981).
3. V. A. Brumberg and L. Z. Pevzner, *Tsitologiya*, **14**, No. 5, 674-676 (1972).
4. M. V. Kudryavtseva, E. E. Zavadskaya, A. D. Skorina, *et al.*, *Lab. Delo*, No. 9, 21-22 (1983).
5. N. N. Mamaev, O. V. Kovaleva, Kh. K. Amineva, *et al.*, *Arkh. Patol.*, No. 3, 43-45 (1993).
6. F. Z. Meerson, *Physiology of Adaptation Processes* [in Russian], Moscow (1986).
7. E. I. Khasina, G. I. Bezdetko, and V. I. Yan'kova, *Byull. Sib. Otdelen. Rossiisk. Akad. Med. Nauk*, No. 1, 51-55 (1998).
8. E. I. Khasina, E. E. Trebukhov, and O. N. Zolotukhina, *Dal'nevostochn. Med. Zh.*, No. 2, 45-48 (2001).
9. S. Edwards, S. Afford, and J. Crocker, *Exp. Cell. Res.*, **194**, No. 1, 118-121 (1991).
10. T. Fukunaga, M. Sasaki, Y. Araki, *et al.*, *Digestion*, **67**, 42-49 (2003).
11. P. Goldin-Lang, G. Dongowski, and H. J. Zunft, *Z. Ernahrungswiss*, **37**, Suppl. 1, 88-91 (1998).
12. M. Neuburger, G. W. Herget, L. Plaumann, *et al.*, *Pathol. Res. Pract.*, **194**, No. 6, 385-389 (1998).
13. N. Papadopoulos, A. Kotini, M. Lambropoulou, and D. Tamiolakis, *Clin. Exp. Obstet. Gynecol.*, **29**, No. 1, 57-61 (2002).