

Peptide bioregulation of aging: results and prospects

Vladimir N. Anisimov · Vladimir Kh. Khavinson

Received: 4 August 2009 / Accepted: 22 September 2009 / Published online: 15 October 2009
© Springer Science+Business Media B.V. 2009

Abstract The review comprises the results of author's long-term investigation in the mechanisms of aging and a role of peptide bioregulators in prevention of age-related pathology. A number of small peptides have been isolated from different organs and tissues and their analogues (di-, tri-, tetrapeptides) were synthesized from the amino acids. It was shown that long-term treatment with some peptide preparations increased mean life span by 20–40%, slow down the age-related changes in the biomarkers of aging and suppressed development of spontaneous and induced by chemical or radiation carcinogens tumorigenesis in rodents. Possible mechanisms of the biological effects of small peptides are discussed in the paper. The results of clinical applications of peptide preparation during the period of 6–12 years are presented as well.

Keywords Small peptides · Pineal gland · Thymus · Life span extension · Tumors · Rodents

Introduction

It is known that specific limit of animal and human lifespan is approximately 30–40% higher than their mean lifespan. It could be referred to the impact of adverse factors causing changes in the gene structure and expression accompanied by disorders in the protein synthesis and organism functioning. The involution of the central organ of immune system—thymus and that of the neuroendocrine system—pineal gland are major manifestations of these disturbances.

To restore functions of thymus, pineal gland and other organs we developed a special method for isolation, refinement and fractionating of low-molecular peptides from extracts of these organs (Khavinson 2004, 2006; Morozov and Khavinson 1991, 1996). Low-molecular peptides isolated from thymus (preparation Thymalin) and pineal gland (preparation Epithalamin) of animals were studied in different biologic models. These peptide preparations contributed to a reliable increase in animals mean lifespan in numerous experiments (Table 1). Of particular importance is a correlation between mean lifespan and the main index of cellular immunity (reaction of blast-transformation of lymphocytes with phytohaemagglutinin) determining T lymphocytes function (Anisimov et al. 1982a, b).

A significant increase in the mean lifespan of animals was evidently caused by a reliable antitumor activity of low-molecular peptides isolated from thymus and pineal gland (Table 2).

V. N. Anisimov (✉)
N.N. Petrov Research Institute of Oncology,
Leningradskaya Str., 68, Pesochny-2,
197758 St. Petersburg, Russia
e-mail: aging@mail.ru

V. Kh. Khavinson
St. Petersburg Institute of Bioregulation and Gerontology,
Prospekt Dynamo, 3, 197110 St. Petersburg, Russia

Table 1 Effect of peptide bioregulators on life span of rodents

Peptide preparations	Species, strain of rodents	Sex	Age at the start of treatment, months	Life span, % to the control		Effect on spontaneous tumor	Reference
				Mean	Maximum		
Epithalamin	Rats	Female	3.5	+25*	+6	↓	Dilman et al. (1979a, 1979b)
		Female	15	+6	+9	↓	Anisimov and Khavinson (1991)
	C3H/Sn mice	Female	3.5	+31*	+14	↓	Anisimov et al. (1982a, 1982b)
		Female	3.5	+13	+5	↓	Anisimov et al. (1987)
	SHR mice	Female	3.5	+11*	-2	↓	Anisimov et al. (1989)
		Female	12	+6	-3	↓	Anisimov et al. (1989)
Thymalin	C3H/Sn mice	Female	3.5	+28*	+11	↓	Anisimov et al. 1982a, 1982b
		Female	3.5	+12*	0	↓	Anisimov et al., 1987
	SHR mice	Female	3.5	-2	-6	↓	Anisimov et al. (1989)
		Female	12	+13*	0	↓	Anisimov et al. (1989)
Glu-Trp	Rats	Female	4	+2	+14	↓	Anisimov et al., 2000
Lys-Glu	Mamm CBA	Female	6	+3*	+7	↓	Anisimov et al. 2001a, 2001b, 2002a, 2002b
Ala-Glu-Asp-Gly	HER-2neu mice	Female	2	-7	0	↑	Anisimov et al. (2002a, 2002b)
		Female	4	-8	-6	↓	Vinogradova et al. (2007)
	SHR mice	Male	4	+7*	+5	↓	Vinogradova et al. (2008)
		Female	3	+13**	12	↓	Anisimov et al. (2003)
	CBA mice	Female	6	+5*	+42	↓	Anisimov et al. (2001a, 2001b, 2002a, 2002b)
		Female	2	+5	-8	=	Anisimov et al. (2005)
SAMP-1 mice	Female	2	+7*	+8	=	Anisimov et al. (2005)	
HER-2neu mice	Female	2	+13*	+14	↓	Anisimov et al. (2002a, 2002b)	
DSIP	SHR mice	Female	3	+17**	+24	↓	Popovich et al. (2003)
	SHR mice	Female	3	+20**	+2	=	Voitenkov et al. (2008)

DSIP delta-sleep inducing peptide, Trp-Ala-Gly-Asp-Als-Ser-Gly-Glu

↓, decrease in the incidence and/or multiplicity and/or increase of tumor latency; =, no effect; *, The difference with the controls is statistically significant, $P < 0.05$

* Mean life span of last 10% survivors

Small peptides isolated from different organs and tissues as well as their synthesized analogues (di-, tri-, tetrapeptides) revealed a reliable tissue specific (gene specific) effects both in cellular cultures and in experimental in young and old animals (Khavinson et al. 1997; Khavinson 2001).

Peptide tissue specific activity manifested in stimulation of protein synthesis in cells of those organs they had been isolated from. The enhancement of protein synthesis under the effect of the peptide has been registered in young and old animals (Brodsky et al. 2001).

Especially significant appeared restoration of reproductive function system in old female rats

subjected to the pineal peptide treatment (Dilman et al. 1979a, b). Persistent estrus and anestrus in rodents, analogous to menopause in women, lowered from the initial 95% down to 52% after the preparation administration, while regular cycles, typical of the normal estrus function, increased from the initial 5% up to 48%. It should be emphasized that in a special experiment initially none of the rats got pregnant after mating. Repeated mating after the administration of the pineal gland peptide entailed pregnancy in 4 out of 16 animals which gave birth to 5–9 healthy off-springs each.

Thus there were ascertained main advantages of low-molecular peptides: they possessed high biologic

Table 2 Effect of peptide bioregulators on induced carcinogenesis in rodents

Peptide preparations	Species, strain of rodents	Carcinogenic agent	Main tumor site	Effect on tumor development	Reference
Epithalamin	Rats	DMBA	Mammary gland	↓	Dilman et al. (1979a, b)
	Rats	NEU, transplacentally	Nervous system, kidney	↓	Alexandrov et al. (1996)
	Rats	X-rays	Malignancies at various sites	↓	Anisimov et al. (1982a, b)
Thymalin	Rats	DMBA	Mammary gland	↓	Anisimov et al. (1980)
	Rats	NEU, transplacentally	Nervous system, kidney	↓	Alexandrov et al. (1996)
	Rats	X-rays	Malignancies at various sites	↓	Anisimov et al. (1982a, b)
	Mice	X-rays	Mammary gland	↓	Anisimov and Khavinson (1993)
Glu-Trip	Rats	⁹⁰ Sr and ¹³⁷ Cs	Mammary gland	↓	Anisimov et al. (1992a, b)
Lys-Glu	Rats	BHBNA	Bladder	↓	Pliss et al. (2001a, b)
	SHR mice	DMH	Colon	↓	Pliss et al. (2005)
Ala-Glu-Asp-Gly	FVB/N mice	HER-2/neu	Mammary gland	↑	Anisimov et al. (2002a, b)
	Rats	Constant light	Tumors at various sites	↓	Vinogradova et al. (2007, 2008)
	Rats	Natural illumination*	Tumors at various sites	↓	Vinogradova et al. (2007)
	Rats	DMH	Colon	↓	Anisimov et al. (2002a, b) and Kossov et al. (2003)
	C3H/He mice	MMTV	Mammary gland	↓	Kossov et al. (2006)
FVB/N mice	HER-2/neu	Mammary gland	↓	Anisimov et al. (2002a, b)	

BHBNA, *N*-butyl-*N*(4-hydroxybutyl)-nitrosamin, DMBA, 7,12-dimethyl(a)anthracene, DMH, 1,2-dimethylhydrazine, NEU, *N*-nitrosoethylurea, HER-2/neu, oncogene, MMTV, murine mammary tumor virus

* Natural light at the Russian North-West (Petrozavodsk)

activity, revealed tissue specificity and were neither species specific nor immunogenic. These features make regulatory peptides similar to peptide hormones.

A detailed study of molecular weight, chemical properties, amino acid composition and sequence of low-molecular peptides isolated from thymus, pineal gland and other organs had been carried out for many years (Morozov and Khavinson 1991, 1996, 1997, 2000). The obtained data were used for chemical synthesis of several small peptides. A comparative analysis showed that biological activity of natural and synthetic preparations was largely identical. Thus, for example, the thymic dipeptide stimulated immunity (Morozov and Khavinson 1996, 2000). Biological activity of natural and synthetic peptides appeared to be similar in standard assays both *in vitro* and *in vivo* (Anisimov et al. 1990, 1998a, b, 2000; Khavinson et al. 1997). These results demonstrate prospects for

application of these peptides as geroprotectors (Anisimov and Khavinson, 2003, 2009; Khavinson and Anisimov 2009). The necessity of searching for new drugs—geroprotectors dictated the onset of preclinical studies of these preparations on different structural levels.

On the level of the organism in different animals we have registered a significant variety of biologic effects exerted by small peptides especially by peptides of thymus and pineal gland, including proliferative activity and apoptosis (Khavinson and Mylnikov 2000a, b; Khavinson et al. 2000a, b, c, 2001a, b; Anisimov et al. 1994, 1997, 2001a, b, 2002a, b; Khavinson 2002).

On the level of cellular structures, small peptides activate heterochromatin in the cell nuclei in senile patients (Lezhava 1984, 2006).

As it was said above there are two forms of chromatin in the cellular nucleus: light euchromatin

and dense heterochromatin located near nuclear membrane. Gene transcription takes place in the light phase, that's in euchromatin. With aging the amount of heterochromatin in the nucleus increases on average from 63 to 80%. Regulatory peptides entail the increase in the amount of euchromatin in the nucleus. This means that more genes become available for transcription factors, and transcription of gene information goes on more intensively as well as protein synthesis. In other words, the more euchromatin there is in the nucleus the more intensive the protein synthesis in the cell is (Lezhava 1984, 2006; Khavinson et al. 2003).

The capability of peptides to induce polypotent cells differentiation is of special significance (Khavinson 2002). Thus addition of retinal peptides to polypotent cells of *Xenopus laevis* early gastrula ectoderm led to the emergence of retinal and pigment epithelium cells. This outstanding result explains a pronounced clinical effect of the preparation of the retina in patients with retinal degenerations (Khavinson 2002) and in animals with genetically determined retinitis pigmentosa (Khavinson et al. 2002b).

On a chromosome level the number of chromosomes aberrations was used a marker of DNA damages in an aging organism. Somatic mutations can occur due to accumulation of stable aberrations and underlie age-related pathology, including malignancy. Reliable antimutagenic and reparative activity of thymus and pineal gland peptides have been confirmed by a reduction in the number of chromosome aberrations in the bone marrow cells and cornea epithelium cells in animals revealing accelerated aging (Anisimov et al. 2004a).

On the level of gene activity regulation it was established that administration of peptides Lys-Glu and Ala-Glu-Asp-Gly to female transgenic HER-2/neu mice caused a 2–3.6-fold suppression of the gene expression as compared to the control group. This suppression is accompanied by a reliable reduction of the tumor diameter (Anisimov et al. 2002a, b).

It was revealed that addition of tetrapeptide Ala-Glu-Asp-Gly to the cultural medium of human lung fibroblasts induced telomerase gene expression and contributed to a 2.4-fold lengthening of telomeres. Activation of gene expression is accompanied by a growing number of cellular divisions (by 42.5%), which is the evidence of Hayflick's limit overcoming (Khavinson et al. 2004a, b).

The effect of treatment with di- and tetrapeptides (Lys-Glu, Glu-Trp, Ala-Glu-Asp-Gly, Ala-Glu-Asp-Pro) on the expression of 15,247 genes was studied with the employment of DNA-microarray technology in heart and brain of CBA mice (Anisimov et al. 2004a, b). In this experiment, there were used clones from the library of the National Institute on Aging, USA. This experiment provided unique data on alteration in the expression of different genes under the effect of peptide preparations. An important conclusion drawn from the experiment was that every peptide specifically regulates particular genes. Results of this experiment testify to the existing mechanism of peptide regulation of gene activity. It was also registered that dipeptide Lys-Glu, showing immunomodulating activity, regulates gene interleukin-2 expression in blood lymphocytes (Khavinson et al. 2000b).

On the molecular level, there was an obvious gap between multiple evidence of specific effects, caused by regulatory peptides in activation of gene transcription (Khavinson et al. 2002a, c; Anisimov and Khavinson 2003; Sibarov et al. 2002; Anisimov et al. 2003; Djeridane et al. 2003; Kossov et al. 2003; Khavinson and Morozov 2003; Labunets et al. 2004a, b), and limited schemes of the process underlying the selective binding of the transcription factor with specific DNA sites. Meanwhile non-specific binding of proteins with the DNA double helix was proved using physicochemical methods (Riadnova et al. 2000). Activation of gene transcription in cells of higher organisms as a rule needs dozens of macromolecular activators and transcription factors.

We proposed a molecular model of interaction between regulatory peptides and DNA double helix in gene promoter region of (Fig. 1a, b; Khavinson et al. 2005, 2006).

Geometrical and chemical complementarity of peptide amino acid sequence and DNA nucleotide pairs sequence was assumed as a basis for the molecular model. Regulatory peptide recognizes a specific site in the DNA double helix if its own amino acid sequence is complementary to the DNA nucleotide sequence for a sufficient length. In other words, their interaction is specific due to matching sequences.

Each sequence of the DNA double helix nucleotide pairs forms a unique pattern of functional groups on the surface of the DNA double helix major groove. A peptide in the unfolded β -conformation can

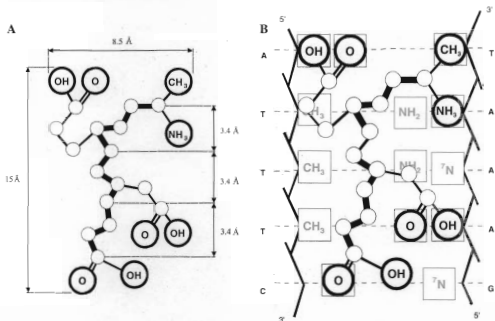


Fig. 1 **a** Unfolded peptide Ala-Glu-Asp-Gly conformation (plan projection). There are shown end and side functional groups, capable of complementary interaction with DNA. $-NH_2$ proton donors groups, $=O$ proton acceptors groups, $-CH_3$

complimentary fit into the in the DNA major groove along the double helix axis. We used data on molecular geometry of the DNA double-helix and peptide β -thread from scientific publications in order to identify nucleotide pairs sequence for specific binding of the DNA and peptide Ala-Glu-Asp-Gly. The screening conducted showed that this tetrapeptide can be located in the DNA major groove with the ATTGG (or ATTTC) nucleotide sequence on the main chain in compliance with the complementarity of disposition of their functional groups (Riadnova et al. 2000).

For experimental testing of the molecular model there were used synthetic preparations: DNA [poly(dA-dT):poly(dA-dT)] (double helix) and tetrapeptide Ala-Glu-Asp-Gly. Gel chromatography helped to prove that peptide Ala-Glu-Asp-Gly forms stable intermolecular complex with the DNA double helix (Khavinson et al. 2006).

Complementary binding of the peptide with nucleotides sequence on the leading strand TATATA

hydrophobic (methyl) group, **Bold line** represents main peptide chain. **b** Scheme of complementary interaction of tetrapeptide Ala-Glu-Asp-Gly with DNA double helix ("DNA-tetrapeptide" complex on the promoter segment of telomerase gene)

of the double helix can be conducted by six hydrogenous and one hydrophobic bonds between functional groups of the both participants.

Under normal physiological conditions DNA exists in the form of a double helix two polymeric chains of which are kept together by hydrogenous bindings between pairs of bases of each chain. Most of the biological processes with DNA participation (transcription, replications) need the double helix to undergo disjunction into separate strands. In particular, it is known, that local separation of double helix strands precedes gene transcription by RNA polymerase. For the transcription onset (synthesis of the matrix RNA) the DNA double helix has to be freed from histones, and in the place where the matrix RNA synthesis starts, the strands of the double helix should be disintegrated.

Concentration dependent hyperchromic effect (increased optic density 260 nm) was found by spectrophotometry of solutions containing synthetic DNA double helix and tetrapeptide Ala-Glu-Asp-Gly.

The hyperchromic effect points out a partial destruction of hydrogen bonds between nucleotide pairs of the double helix and local separation of its strands (allosteric conformational changes; Khavinson et al. 2006). In vitro experiments show that a small peptide of the definite structure and amino acid sequence can participate in activation of genes transcription on the stage of strands disjunction in the DNA double helix. Biochemical aspect of this phenomenon consists in similarity of structure and amino acid sequence of a regulatory peptide and a specific segment of the peptide chain of the macromolecular transcription factor.

Thus, the studies of peptides biological activity on different structural levels and of physicochemical processes of their interaction proved an indubitably high physiologic activity of peptide regulators. Major conclusion reads that peptides are capable of regulating gene expression. Pre-clinical trials demonstrated high biological activity and safety of synthesized peptides (Khavinson 2001; Khavinson et al. 2001a, b, c, 2002a, b, c, d, 2006). Thus, the administration of peptides Lys-Glu or Ala-Glu-Asp-Gly to animals contributes to a reduced incidence of tumors and an increase of mean lifespan (Tables 1, 2). Peptide Ala-Glu-Asp-Pro stimulates nerve regeneration (Turchaninova et al. 2000), peptide Lys-Glu-Asp-Trp decreases blood glucose level in animals with experimental diabetes mellitus (Khavinson et al. 2007a, b, c).

Taking into consideration a reliable biological activity of peptides we found it reasonable to study

the effect of regulatory peptides in monkeys. Restoration of the melatonin level up to normal following the administration of the peptide preparation to old monkeys was among our significant findings. The same old monkeys revealed a restoration to normal indices of a daily rhythm of secretion of the main hormone of adrenal gland—cortisol (Khavinson et al. 2001a, b, c).

Effect of the peptide bioregulators in human

Taking into consideration the encouraging data testifying to high geroprotective activity of both natural tissue specific and synthetic peptide preparations we have been concentrating our attention on studies of geroprotective activity of peptides in old and senile people in recent years (Khavinson 2002; Khavinson and Morozov 2003; Labunets et al. 2004a, b; Korkushko et al. 2004; Anisimov and Khavinson 2005; Khavinson and Malinin 2005; Goncharova et al. 2005; Kozina et al. 2007). Thus, annual treatment course with thymus and pineal preparations led to a reliable decrease in mortality (Table 3), to improvement of brain function and that of immune, endocrine, cardio-vascular systems, increased density of osseous tissue (Khavinson 2002; Khavinson and Morozov 2003; Labunets et al. 2004a, b; Korkushko et al. 2004). It is noteworthy that application of preparation of the thymus led to a twofold decrease in frequency of acute respiratory disease (Khavinson and Morozov 2003). The restoration of nocturnal

Table 3 Effect of treatment with peptide preparation on mortality rate in elderly and old subjects

Group of subjects	Indices	Control (administration of polyvitamin)	Administration of the pineal gland preparation	Administration of the complex of thymus and pineal gland preparations
Elderly people (60–74 years)	Number of subjects	48	46	No studies
	Initial mean age (years)	69.3 ± 2.2	71.1 ± 1.4	
	Mortality rate in the course of 8 years (%)	13.6	8.5*	
	Mortality rate in the course of 12 years (%)	44.1	22.3*	
Old people (75–89 years)	Number of subjects	22	24	20
	Initial mean age (years)	80.2 ± 1.6	81.5 ± 2.1	82.1 ± 2.3
	Mortality rate in the course of 6 years (%)	81.8	45.8*	33.3*

* $P < 0.05$ as compared to the control

melatonin secretion level in patients subjected to administration of preparation of the pineal gland is of special significance (Labunets et al. 2004a, b). These results suggest good prospects for tackling demographic issues (Khavinson and Mikhailova 2007).

Conclusion

Involvement of the main organs and tissues of the organism accompanied by a decrease of protein synthesis in cells underlies the process of aging. Peptide preparations isolated from organs of young animals when administered into an old organism are capable to induce protein synthesis followed by restoration of the main functions.

It was shown that long-term application of peptides, both isolated from the organs and synthesized from the amino acid, in animals (as a rule starting from the period after maturity) leads to a reliable increase in their mean lifespan by 20–40% and reaching a species limit.

It was revealed that small peptides (di-, tri- and tetrapeptides) are capable of complementary interaction with the DNA specific binding site on the

promoter segment of genes, inducing disjoining of double helix strands and RNA polymerase activation. Discovery of the phenomenon of peptide activation of gene transcription points out the natural mechanism of organism to maintain physiologic functions, which is based on the complementary interaction of the DNA and regulatory peptides. This process is fundamental for the development and functioning of the living substance (Figs. 2, 3), while aging is an evolutionary determined biologic process of age-related changes in gene structure and expression.

Application of peptide bioregulators in humans for preventive purposes led to a significant rehabilitation of the main physiological functions and a reliable mortality decrease in different age groups during the period of 6–12 years.

It should be emphasized that this approach to the prevention of aging is based not only on experimental and clinical data, but also on technological developments having world novelty (Anisimov 2008; Khavinson et al. 2006, 2007a, b, 2008a, b).

The authors and their teams believe that the whole complex of 35-year experimental and clinical studies may serve a vital contribution to the advancement of a scientific heritage of the prominent Russian

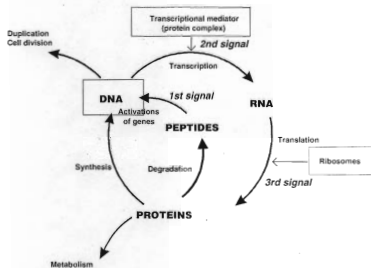


Fig. 2 The role of peptides in the cycle of DNA, RNA and protein biosynthesis

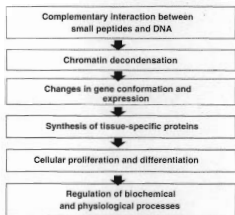


Fig. 3 Mechanism of peptide regulation of the living matter development

scientist I. I. Mechnikov in the field of gerontology and will be to the benefit of people, especially for those of old and oldest old age.

Acknowledgments The author expresses his sincere gratitude to our colleagues from the St. Petersburg Institute of Bioregulation and Gerontology and from another research institutes over Russia, Ukraine and Belorussia as well as foreign colleagues Professors J. Atzpodien (Germany), K. R. Boheler (USA), C. Franceschi (Italy), E. Lakatta (USA), T. A. Lezhava (Georgia), J. Martinez (France), M. Passeri (Italy), Y. Toulitou (France), A. I. Yashin (USA), I. Zisman (Israel) for their significant contribution into our research work.

References

- Alexandrov VA, Beshpalov VG, Morozov VG, Khavinson VKh, Anisimov VN (1996) Study of the postnatal effects of chemopreventive agents on ethylnitrosourea-induced transplacental carcinogenesis in rats. II. Influence of low-molecular-weight polypeptide factors from the thymus, pineal glands, bone marrow, anterior hypothalamus, brain cortex and brain white substance. *Carcinogenesis* 17: 1931–1934
- Anisimov VN (2008) Molecular and physiological mechanisms of aging, vol 1 & 2. Nauka Publ, St. Petersburg
- Anisimov VN, Khavinson VKh (1991) Effect of polypeptide pineal preparation on life span and spontaneous tumor incidence in the elderly female rats. *Dokl Akad Nauk SSSR* 319:250–253
- Anisimov VN, Khavinson VKh (1993) Using of polypeptide pineal preparation in oncology: twenty-year experience of studies on epithalamine. *Vopr Onkol* 39:131–142
- Anisimov VN, Khavinson VKh (2003) Small peptide-associated modulation of aging and longevity. In: Rattan SIS (ed) *Modulating aging and longevity*. Kluwer, Dordrecht, pp 279–301
- Anisimov VN, Khavinson VKh (2005) Pineal peptides as modulators of aging. In: Rattan SIS (ed) *Aging interventions and therapies*. World Scientific, Singapore, pp 127–146
- Anisimov VN, Khavinson VKh (2009) Use of peptide bioregulators for cancer prevention. Results of 35-year research and prospects. *Vopr Onkol* 55:291–304
- Anisimov VN, Danetskaya EV, Morozov VG, Khavinson VKh (1980) Inhibitory effect of polypeptide thymic factor on development of mammary adenocarcinomas induced by 7, 12-dimethylbenz(a)anthracene in female rats. *Dokl Akad Nauk SSSR* 250:1485–1487
- Anisimov VN, Khavinson VKh, Morozov VG (1982a) Carcinogenesis and aging. IV. Effect of low-molecular-weight factors of thymus, pineal gland and anterior hypothalamus on immunity, tumor incidence and lifespan of C3H/5n mice. *Mech Aging Dev* 19:245–258
- Anisimov VN, Mireski GI, Morozov VG, Khavinson VKh (1982b) Effect of polypeptide factors of thymus and pineal gland on radiation carcinogenesis. *Bull Exp Biol Med* 94:80–82
- Anisimov VN, Morozov VG, Khavinson VKh (1987) Effect of polypeptide factors of thymus, bone marrow, pineal gland and vessels on life span and tumor development in mice. *Dokl Acad Nauk SSSR* 293:1000–1004
- Anisimov VN, Loktionov AS, Khavinson VKh, Morozov VG (1989) Effect of low-molecular-weight factors of thymus and pineal gland on lifespan and spontaneous tumour development in female mice of different age. *Mech Aging Dev* 49:245–257
- Anisimov VN, Bondarenko LA, Khavinson VKh (1990) The pineal peptides: interaction with indoles and the role in aging and cancer. In: Gupta D, Wollmann HA, Rankin MB (eds) *Neuro-endocrinology: new frontiers*. Brain Research Promotion, London–Tübingen, pp 317–325
- Anisimov VN, Bondarenko LA, Khavinson VKh (1992a) Effect of pineal peptide preparation (epithalamine) on lifespan and pineal and serum melatonin level in old rats. *Ann NY Acad Sci* 673:53–57
- Anisimov VN, Mireski GI, Morozov VG, Praveleva IA, Khavinson VKh (1992b) Effect of synthetic immunomodulator thymogen on radiation carcinogenesis in rats. *Vopr Onkol* 38:451–458
- Anisimov VN, Khavinson VKh, Morozov VG (1994) Twenty years of study on effect of pineal peptide preparation: epithalamine in experimental gerontology and oncology. *Ann NY Acad Sci* 719:483–493
- Anisimov VN, Mylnikov SV, Oparina TI, Khavinson VKh (1997) Effect of melatonin and pineal peptide preparation epithalamine on lifespan and free radical oxidation in *Drosophila melanogaster*. *Mech Aging Dev* 97:81–91
- Anisimov VN, Khavinson VKh, Morozov VG (1998a) Effect of synthetic dipeptide Thymogen® (Cis-Trp) on lifespan and spontaneous tumor incidence in rats. *Gerontologist* 38:7–8
- Anisimov VN, Mylnikov SV, Khavinson VKh (1998b) Pineal peptide preparation epithalamine increases the lifespan of fruit flies, mice and rats. *Mech Aging Dev* 103:123–132

- Anisimov VN, Khavinson VKh, Morozov VG (2000) Immunomodulatory peptide L-Glu-L-Trp slows down aging and inhibits spontaneous carcinogenesis in rats. *Biogerontology* 1:55–59
- Anisimov VN, Arutjunyan AV, Khavinson VKh (2001a) Effects of pineal peptide preparation Epithalamin on free-radical processes in humans and animals. *Neuro Endocrinol Lett* 22:9–18
- Anisimov VN, Khavinson VKh, Mikhalski AI, Yashin AI (2001b) Effect of synthetic thymic and pineal peptides on biomarkers of aging, survival and spontaneous tumour incidence in female CBA mice. *Mech Aging Dev* 122:41–68
- Anisimov VN, Khavinson VKh, Provinciali M, Alimova IN, Baturin DA, Popovich IG, Zabezhinski MA, Imyanitov EN, Mancini R, Franceschi C (2002a) Inhibitory effect of the peptide epithalon on the development of spontaneous mammary tumors in Her-2/NEU transgenic mice. *Int J Cancer* 101:7–10
- Anisimov VN, Khavinson VKh, Popovich IG, Zabezhinski MA (2002b) Inhibitory effect of peptide Epitalon on colon carcinogenesis induced by 1, 2-dimethylhydrazine in rats. *Cancer Lett* 183:1–8
- Anisimov VN, Khavinson VKh, Popovich IG, Zabezhinski MA, Alimova IN, Rosenfeld SV, Zavarzina NYu, Semchenko AV, Yashin AI (2003) Effect of epithalon on biomarkers of aging, lifespan and spontaneous tumor incidence in female Swiss-derived SHR mice. *Biogerontology* 4:193–202
- Anisimov SV, Boheler KR, Khavinson VKh, Anisimov VN (2004a) Elucidation of the effect of brain cortex tetrapeptide Cortagen on gene expression in mouse heart by microarray. *Neuro Endocrinol Lett* 25:87–93
- Anisimov VN, Popovich IG, Zabezhinski MA, Rosenfeld SV (2004b) Spontaneous mutagenesis, carcinogenesis and aging in SAM mice: effect of melatonin, epithalon and neuronal. In: Takeda T (ed) Proceedings of the 19th SAM meeting, Kyoto, pp 101–102
- Brodsky VY, Khavinson VKh, Zolotarev YuA (2001) Rhythm of protein synthesis in cultures of hepatocytes of rats at different age. *Izv Akad Nauk Ser Biol* 5:517–521
- Dilman VM, Anisimov VN, Ostroumova MN, Khavinson VKh, Morozov VG (1979a) Increase in lifespan of rats following polypeptide pineal extract treatment. *Exp Pathol* 17:539–545
- Dilman VM, Anisimov VN, Ostroumova MN, Morozov VG, Khavinson VKh, Azarova MA (1979b) Study of the anti-tumor effect of polypeptide pineal extract. *Oncology* 36:274–280
- Djeridane Y, Khavinson VKh, Anisimov VN, Toulitou Y (2003) Effect of synthetic pineal tetrapeptide (Ala-Glu-Asp-Gly) on melatonin secretion by the pineal gland of young and old rats. *J Endocrinol Invest* 26:211–215
- Goncharova ND, Vengerin AA, Khavinson VKh, Lapin BA (2005) Pineal peptides restore the age-related disturbances in hormonal functions of the pineal gland and the pancreas. *Exp Gerontol* 40:51–57
- Khavinson VKh (2001) Tissue-specific effects of peptides. *Bull Exp Biol Med* 132:807–808
- Khavinson VKh (2002) Peptides and aging. *Neuro Endocrinol Lett*—special issue
- Khavinson VKh (2004) US Patent N 6,727,227 B1 "Tetra-peptide revealing geroprotective effect, pharmacological substance on its basis, and the method of its application", 27 April 2004
- Khavinson VKh (2006) US Patent N 7,101,854 B2 "Tetra-peptide stimulating the functional activity of hepatocytes, pharmacological substance on its basis and the method of its application", 5 Sept 2006
- Khavinson VKh, Anisimov VN (2009) 35-years experience in study of peptide regulation of aging. *Adv Gerontol* 22:11–23
- Khavinson VKh, Malinin VV (2005) Gerontological aspects of genome peptide regulation. *Karger AG, Basel (Switzerland)*
- Khavinson V, Morozov V (2003) Peptides of pineal gland and thymus prolong human life. *Neuro Endocrinol Lett* 24:233–240
- Khavinson VKh, Mikhailova ON (2007) Health and aging in Russia. In: Robinson M (ed) *Global health and global aging*. Josse-Bass, San Francisco, pp 226–237
- Khavinson VKh, Mylnikov SV (2000a) Effect of Epitalon on the age-specific changes in the time course of lipid peroxidation in *Drosophila melanogaster*. *Bull Exp Biol Med* 130:1116–1119
- Khavinson VKh, Mylnikov SV (2000b) Effect of pineal tetrapeptide on antioxidant defense in *Drosophila melanogaster*. *Bull Exp Biol Med* 129:355–356
- Khavinson VKh, Chalisova NI, Okulov VB (1997) The neuropeptide-stimulating effect of peptides from brain in dorsal root ganglion neuron organotypic culture. *Prim Sensory Neuron* 2:191–200
- Khavinson VKh, Anisimov VN, Zavarzina NYu, Zabezhinskii MA, Zimina OA, Popovich IG, Shelyk AV, Malinin VV, Morozov VG (2000a) Effect of vilon on biological age and lifespan in mice. *Bull Exp Biol Med* 130:687–690
- Khavinson VKh, Izmailov DM, Obukhova LK, Malinin VV (2000b) Effect of epithalon on the lifespan increase in *Drosophila melanogaster*. *Mech Aging Dev* 120:141–149
- Khavinson VKh, Morozov VG, Malinin VV, Kazakova TB, Korneva EA (2000c) Effect of peptide Lys-Glu on Interleukin-2 gene expression in lymphocytes. *Bull Exp Biol Med* 130:898–899
- Khavinson VKh, Goncharova N, Lapin B (2001a) Synthetic tetrapeptide epithalon restores disturbed neuroendocrine regulation in senescent monkeys. *Neuro Endocrinol Lett* 22:251–254
- Khavinson VKh, Morozov VG, Anisimov VN (2001b) Experimental studies of the pineal gland preparation Epithalamin. In: Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke D (eds) *The pineal gland and cancer*. Springer-Verlag, Berlin, pp 294–306
- Khavinson VKh, Mylnikov SV, Oparina TI (2001c) Effect of peptides on generation of reactive oxygen species in subcellular fractions of *Drosophila melanogaster*. *Bull Exp Biol Med* 132:682–685
- Khavinson VKh, Egorova VV, Timofeeva NM, Malinin VV, Gordova LA, Gromova LV (2002a) Effect of vilon and epithalon on glucose and glycine absorption in various regions of small intestine in aged rats. *Bull Exp Biol Med* 133:494–496
- Khavinson VKh, Korneva EA, Malinin VV, Rybakina EG, Pivanovich IYu, Shanin SN (2002b) Effect of epithalon on interleukin-1 β signal transduction and the reaction of

- thymocyte blast transformation under stress. *Neuro Endocrinol Lett* 23:411–416
- Khavinson V, Razumovsky M, Trofimova S, Grigorian R, Razumovskaya A (2002c) Pinealet-regulating tetrapeptide epitaleon improves eye retina condition in retinitis pigmentosa. *Neuro Endocrinol Lett* 23:365–368
- Khavinson VKh, Rybakina EG, Malinin VV, Pivanovich IYu, Shanin SN, Korneva EA (2002d) Effects of short peptides on thymocyte blast transformation and signal transduction along the sphingomyelin pathway. *Bull Exp Biol Med* 133:497–499
- Khavinson VKh, Lezhava TA, Monaselidze JR, Khokhade TA, Dvalis NA, Babishvili NK, Trofimova SV (2003) Peptide Epitalon activates chromatin at the old age. *Neuro Endocrinol Lett* 24:329–333
- Khavinson V, Bondarev I, Butyugov A (2004a) Epitalon peptide induces telomerase activity and telomere elongation in human somatic cells. *Bull Exp Biol Med* 135:590–592
- Khavinson V, Bondarev I, Butyugov A, Smirnova T (2004b) Peptide promotes overcoming of the division limit in human somatic cell. *Bull Exp Biol Med* 137:613–616
- Khavinson V, Shataeva L, Chernova A (2005) DNA double-helix binds regulatory peptides similarly to transcription factors. *Neuro Endocrinol Lett* 26:237–241
- Khavinson VKh, Soloviov AYu, Shataeva LK (2006) Molecular mechanism of interaction between oligopeptides and double-stranded DNA. *Bull Exp Biol Med* 141:457–461
- Khavinson VKh, Malinin VV, Grigoriev EI, Ryzhak GA (2007a) LV Patent N 13462 "Tetrapeptide regulating blood glucose level in diabetes mellitus", 20 Feb 2007
- Khavinson VKh, Morozov VG, Malinin VV, Grigoriev EI (2007b) US Patent N 7,189,701 B1 "Tetrapeptide stimulating the functional activity of neurons, pharmacological agent based thereon and method of use thereof", 13 March 2007
- Khavinson VKh, Gavrishcheva NA, Malinin VV, Chufu SG, Trofimov EL (2007c) Effect of Pancregen on blood glucose level, capillary permeability and adhesion in rats with experimental diabetes mellitus. *Bull Exp Biol Med* 144:559–562
- Khavinson VKh, Ryzhak GA, Grigoriev EI, Ryadnova IYu (2008a) EP Patent N 1 758 922 B1 "Peptide substance restoring function of respiratory organs", 13 Feb 2008
- Khavinson VKh, Ryzhak GA, Grigoriev EI, Ryadnova IYu (2008b) EP Patent N 1 758 923 B1 "Peptide substance restoring myocardium function", 13 Feb 2008
- Korkushko OV, Khavinson VKh, Shatilov VB, Magdich LV (2004) Effect of peptide preparation epithalamin on circadian rhythm of epiphyseal melatonin-producing function in elderly people. *Bull Exp Biol Med* 137:389–391
- Kossov G, Zandbank J, Tendler E, Anisimov VN, Khavinson VKh, Popovich IG, Zabezhinski MA, Zusman I, Ben-Hur H (2003) Epitalon and colon carcinogenesis in rats: proliferative activity and apoptosis in colon tumors and mucosa. *Int J Mol Med* 12:473–477
- Kossov G, Anisimov VN, Ben-Hur H, Kossov N, Zusman I (2006) Effect of the synthetic pineal peptide epitaleon on spontaneous carcinogenesis in female C3H/He mice. *In Vivo* 20:253–257
- Kozina LS, Arutjunyan AV, Khavinson VKh (2007) Antioxidant properties of geroprotective peptides of the pineal gland. *Arch Gerontol Geriatr Suppl* 1:213–216
- Kvetnoy IM, Reizer RJ, Khavinson VKh (2000) Claude Bernard was right: hormones may be produced by "non-endocrine" cells. *Neuro Endocrinol Lett* 21:173–174
- Labunets IF, Butenko GM, Khavinson VKh (2004a) Effect of bioactive factors of the pineal gland on thymus function and cell composition of the bone marrow and spleen in mice of different age. *Bull Exp Biol Med* 137:620–622
- Labunets IF, Butenko GM, Magdich LV, Korkushko OV, Khavinson VKh, Shatilov VB (2004b) Effect of epithalamin on circadian relationship between the endocrine function of the thymus and melatonin-producing function of the pineal gland in elderly people. *Bull Exp Biol Med* 137:617–619
- Lezhava T (1984) Heterochromatinization as a key factor in aging. *Mech Aging Dev* 28:279–288
- Lezhava T (2006) Human chromosomes and aging. From 80 to 114 years. Nova Biomedical, New York
- Morozov VG, Khavinson VKh (1991) US Patent N 5,070,076 "Thymus-Gland preparation and method for producing same", 03 Dec 1991
- Morozov VG, Khavinson VKh (1996) US Patent N 5,538,951 "Pharmaceutical preparation for the therapy of immune deficiency conditions", 23 July 1996
- Morozov VG, Khavinson VKh (1997) Natural and synthetic thymic peptides as therapeutics for immune dysfunction. *Int J Immunopharmacol* 19:501–505
- Morozov VG, Khavinson VKh (2000) US Patent N 6,136,788 "Pharmaceutical preparation for the therapy of immune deficiency conditions", 24 Oct 2000
- Pliss GB, Melnikov AS, Malinin VV, Khavinson VKh (2001a) Inhibitory effect of peptide vilon on the development of induced rat urinary bladder tumors in rats. *Bull Exp Biol Med* 131:558–560
- Pliss GB, Melnikov AS, Malinin VV, Khavinson VKh (2001b) Effect of vilon and epitaleon on development of induced bladder tumors in rats. *Vopr Onkol* 47:601–607
- Pliss GB, Melnikov AS, Malinin VV, Khavinson VKh (2005) Effect of vilon (Lys-Glu) on neoplasia induced by 1,2-dimethylhydrazine in mice. *Vopr Onkol* 51:466–469
- Popovich IG, Voitenkov BO, Anisimov VN, Ivanov VT, Mikhaleva IL, Zabezhinski MA, Alimova IN, Baturin DA, Zavarzina NYu, Rosenfeld SA, Semchenko AV, Yashin AI (2003) Effect of delta-sleep inducing peptide-containing preparations Deltaron on biomarkers of aging, life span and spontaneous tumor incidence in female SHR mice. *Mech Aging Dev* 124:721–731
- Riadnova IYu, Shataeva LK, Khavinson VKh (2000) DNA-protein interaction studied in model systems. *Polym Sci Ser A* 42:551–556
- Siburov DA, Kovalevko RI, Malinin VV, Khavinson VKh (2002) Epitalon influences pineal secretion in stress-exposed rats in the daytime. *Neuro Endocrinol Lett* 23:452–454
- Turchaninova LN, Kolosova LI, Malinin VV, Moiseeva AB, Nozdachev AD, Khavinson VKh (2000) Effect of tetrapeptide Cortagen on regeneration of sciatic nerve. *Bull Exp Biol Med* 130:1172–1174

- Vinogradova IA, Bukalev AV, Zabezhinski MA, Semchenko AV, Khavinson VKh, Anisimov SV (2007) Effect of Ala-Glu-Asp-Gly peptide on lifespan and development of spontaneous tumors in female rats exposed to different illumination regimens. *Bull Exp Biol Med* 144:825–830
- Vinogradova IA, Bukalev AV, Zabezhinski MA, Semchenko AV, Khavinson VKh, Anisimov SV (2008) Geroprotective effect peptide Ala-Glu-Asp-Gly in male rats maintained at various regimens of illumination. *Bull Exp Biol Med* 145:455–460
- Voitenkov VB, Popovich IG, Arutiunian AV, Oparina TI, Prokopenko VM (2008) Effect of delta-sleep inducing peptide on free-radical processes in the brain and liver of mice during various light regimens. *Adv Gerontol* 21(1): 53–55