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INVITED NEL REVIEW

Effects of pineal peptide preparation Epithalamin on free-radical processes in humans and animals

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Abstract

OBJECTIVES: The review on our own data on the effect of the pineal peptide preparation Epithalamin on free radical processes in rodents and humans is presented in this paper.

RESULTS: The activity of Cu, Zn-superoxide dismutase (SOD) was found decreased in the brain of aged rats (30 months old) by 46.8% as compared to young animals. Concentration of Schiff's bases in the brain also went down with age (by 13.6%), while the level of dien conjugates (DC) and protein peroxidation (PPO) remained unchanged. General antioxidation activity (AOA) in the brain also remained stable with age. The liver of aged rats showed significant increase of Schiff's bases (by 27.1%) and PPO products (by 109.2%) and considerable decrease of SOD activity. The level of DC and general AOA in the liver remained unchanged with age. Considerable elevation of protein and lipid peroxidation products contents was registered in the blood serum of aged rats. At the same time, general AOA and SOD activity remarkably decreased. The results obtained evidence from both significant age-related alterations in the activity of free radical processes in animal organism and organic peculiarities of their dynamics. Application of peptide drug epithalamin suppressed significantly the intensity of peroxide chemoluminescence in the blood serum (2.8-fold) and lipid peroxide oxidation (LPO) expressed in the considerably decreased DC contents (4,1-fold). The contents of Schiff's bases showed only a tendency towards decrease (by 14.4%, $p>0.05$) and PPO level remained unchanged. Epithalamin administration was followed by considerable (by 36.6%, $p<0.01$) increase of general AOA and increased SOD activity (by 19.7%) in males. Epithalamin decreased significantly the contents of conjugated hydroperoxides and ketodienes in tissues of D.melanogaster females, increased catalase activity in drosophila males and females, and increased SOD activity in males of D.melanogaster by 41%. Humans reveal significant age-related decrease of antioxidation defence indices.

CONCLUSION: Epithalamin administration to patients with age-related pathology eliminates imbalance in prooxidation and antioxidation systems.

Introduction

Studies conducted over the last 25 years have documented a wide range of the biological activity of epithalamin®, a low molecular weight peptide preparation obtained from the pineal gland [1–5]. Treatment of aged animals with epithalamin has been found to normalize some endocrine and immune parameters and other bodily functions. Upon continuous administration, epithalamin appeared to increase the life span of rats, mice, and D. melanogaster fruit flies [2, 6–8], delay reproductive failure and immune dysfunctions in aged animals, and inhibit the development of spontaneous and induced tumors of different locations [2, 4, 6, 8]. The effects produced by Epithalamin seem to be mediated, at least partly, by its ability to stimulate synthesis and secretion of melatonin by the pineal gland and by its modulating influences on immune and some metabolic functions, which suggests an important homeostatic role of pineal peptides [1–4]. It has been found recently that melatonin is one of the most potent endogenous antioxidants [9–11]. This prompted us to conduct a comparative study of the antioxidant properties of epithalamin and melatonin in rats, which revealed that epithalamin is superior to melatonin as an *in vivo* antioxidant [12, 13, 14].

One of the most fruitful theoretical advances in basic gerontology is the free-radical theory of aging. According to this theory, the so-called reactive oxygen species (ROS), including superoxide ($\bullet O2^-$) and hydroxyl ($\bullet HO$) free radicals, hydrogen peroxide (H_2O_2) and, possibly, singlet oxygen ($\uparrow O2$) generated as by products of cellular respiration and other metabolic processes, make damage to cellular macromolecules (DNA, proteins, and lipids) culminating in mutations and genome instability, which leads to the development of age-associated pathological phenomena, including cancer, circulatory diseases, immunodepression, brain dysfunctions, cataract, and others [15–18]. It is still not clear how free radical reactions do cause aging. They are believed, in particular, to damage cell membranes, intercellular matrix, chromatin, and DNA and to contribute to impairments of calcium homeostasis [15, 17–20]. It should be noted that aging is associated with the slowing down of processes that result in free-radical generation. However, antioxidant defenses become less reliable with aging, which can facilitate the damage made by free radicals to tissues [18, 19].

Available data about age-associated changes in free-radical processes are rather discrepant [15, 18, 21, 22]. Therefore, it was, first, necessary to study these changes in model animals, i. e., rats, to evaluate the antioxidant activity of epithalamin and melatonin. The data obtained justify further studies of the effects of epithalamin on free-radical processes in humans.

Age-associated changes in free-radical processes in animals

Data on age-associated changes in the activities of Cu, Zn-superoxide dismutase (SOD) and peroxidation of lipids (LPO) and proteins in different tissues are contradictory [15, 17–19, 23, 24], which may be caused by differences in study objects (species-, strain-, and organ-dependent differences), diets used in different laboratories to feed animals (dietary contents of pro- and antioxidants), and age periods compared. We studied some indicator parameters of free-radical processes and antioxidant defenses in the brain, liver and blood serum of young (3 months) and aged (30 months) rats [25].

As seen from data presented in Table 1, SOD activity was significantly lower (by 46.8%, $p < 0.001$) in the brain of aged rats compared with young rats. The contents of Schiff bases in the brain was somewhat decreased in aged animals (by 13.6%, $p<0.001$), whereas the levels of diene conjugates and protein peroxidation products were unchanged. The total antioxidant activity of brain did not decrease with increased age. In the liver of aged rats, significant increases in Schiff base and protein peroxidation product contents (by 27.1%, $p<0.05$, and by 109.2%, $p<0.01$, respectively) were observed, whereas SOD activity decreased by 58.4%, $p<0.001$). Diene conjugates and the total antioxidant activity did not change in the liver with increased age. A different pattern of age-dependent changes was observed in blood serum. Products of lipid and protein peroxidation were significantly increased in aged rat serum, whereas the total antioxidant and SOD activities were decreased. These results conform to human data showing that the content of glutathione in blood plasma of aged humans (60–97 years) was significantly lower, and the contents of LPO products were increased in comparison with young people (20–39 years) [26].

It is also noteworthy that in either, the liver and blood serum, generation of ROS significantly decreases with increasing age. As noted above, the apparent increase of ROS generation, which occurs in aging, has been shown by a number of authors to be associated with a significant decrease in the activity of antioxidant defenses. This is clearly seen in our experiments: the decrease of ROS production in blood serum and liver is associated with a sharp drop of the activity of SOD, an important component of the enzymic branch of the antioxidant defenses, which inhib-

Table 1. Parameters of free radical processes in the brain, liver, and blood serum of young (3 months) and aged (30 months) male rats [25]

Parameter	Young rats (n = 8)			Old rats (n = 5)		
	Brain	Liver	Serum	Brain	Liver	Serum
ROS (units per mg protein)	N.D.	980,0 ± 122,5	0,34 ± 0,03	4,14 ± 0,14	176,9 ± 34,9***	0,19 ± 0,02**
Diene conjugates (nmole per mg protein)	40,19 ± 2,55	55,8 ± 4,1	3,59 ± 0,32	44,89 ± 1,62	53,20 ± 2,20	5,54 ± 0,34***
Schiff bases (units per mg protein)	318,0 ± 2,9	520,1 ± 26,2	19,0 ± 1,72	274,8 ± 13,7**	661,3 ± 31,1*	25,2 ± 1,74*
Protein peroxidation products (μmole carbonylated amino acids per mg protein)	7,53 ± 0,40	2,39 ± 0,21	1,53 ± 0,06	7,41 ± 0,34	5,0 ± 0,27**	1,86 ± 0,08***
AOA (units per mg protein)	6,34 ± 0,34	14,57 ± 0,71	1,74 ± 0,15	5,89 ± 0,21	14,18 ± 1,19	1,38 ± 0,06*
SOD activity (units per mg protein)	46,6 ± 2,0	116,0 ± 8,5	1,25 ± 0,24	24,8 ± 2,8***	48,3 ± 6,4***	0,85 ± 0,04

Note: The number of analyses is indicated in brackets. For each analysis tissues of three rats were pooled. N.D. – not detected. Statistical significance of differences vs. young animals: * $p<0.05$; ** $p<0.01$; *** $p>0.001$.

its the early steps of generation of ROS and other reactive free radicals. The level of ROS in our studies was determined by H_2O_2–induced chemi-luminescence which is considered to be an appropriate method of ROS estimation [27].

The most vulnerable target of free radicals seems to be proteins, as reflected by their increased peroxidation, which, in the present study, was observed in the liver and blood serum. Other authors have also noticed that, in aging, peroxidation primarily affects proteins, even more so than lipids [28, 29]. However, this was not evident in the aging brain, which, in our view, might be associated, to some extent, with the high activity of low molecular antioxidants in the brain, since the total antioxidant activity in the brain did not change, while SOD activity was suppressed.

It should be noted that the activity of brain cytochrome oxidase decreases with aging [28], which suggests the increased superoxide generation, owing to the fact that the brain is the most aerobic organ with the greatest oxygen consumption per unit mass. Therefore, the brain is more vulnerable to damage caused by oxidants than other organs are and so it needs a pool of low molecular antioxidants, which can be readily replenished.

On the whole, the above data suggest that significant changes occur in the free radical processes in an aging animal body and that these changes are organ-specific (Fig. 1). Products of lipid and protein peroxidation increased in the liver and blood plasma but not in the brain. SOD activity decreased in all organs of aged rats, whereas the total antioxidant activity, which reflects primarily the sum of concentrations of low molecular antioxidants (ascorbic acid, α-tocopherol, uric acid and thiol compounds, mainly glutathione), decreased in blood serum only and was constant in the brain and liver.

The conclusion about differential age-dependencies of free-radical processes in different organs seems important and conforms observations of other authors [15–17, 19, 22] and data about differential sensitivity of body organs to a number of damaging factors, including chemical carcinogens and ionizing radiation [30]. Recently it has been shown using mice made transgenic for LacZ gene that the rates of accumulation of somatic mutations in different murine organs may be quite different, in particular, the rate is much higher in the liver than in the brain [31]. The results of our studies suggest that these differences may be caused not only by differential efficiencies of DNA repair [31] but, also, by organ-specific characteristics of free-radical processes and antioxidant defenses. Also, our results justify the use of antioxidants as geroprotectors and means to prevent the development of age-related pathological conditions.

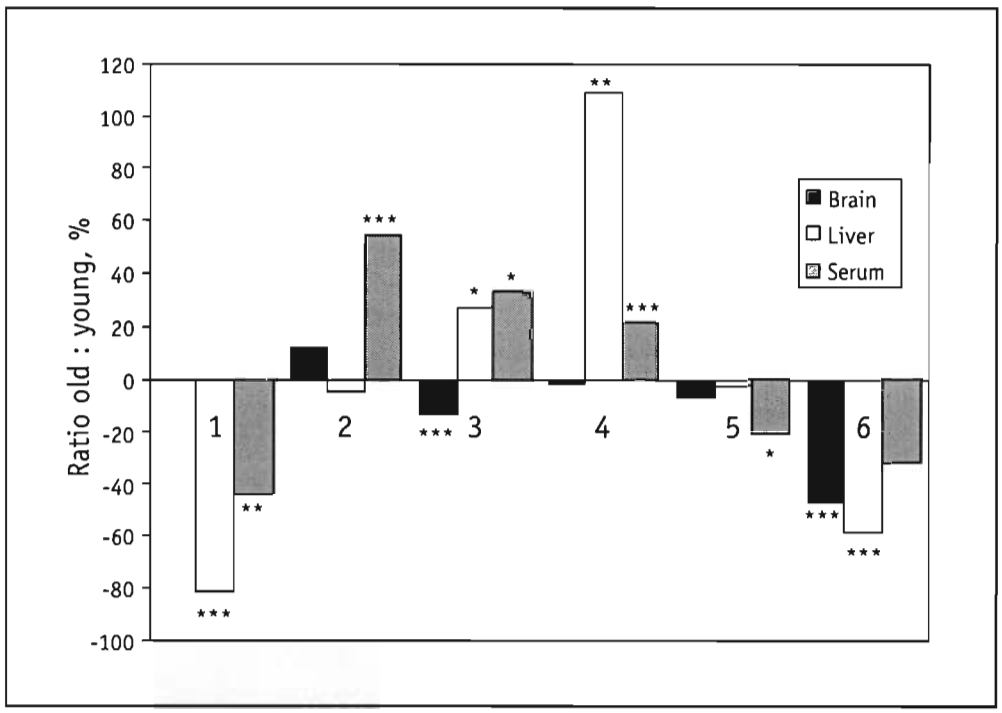

Fig.1. Parameters of free radical processes in tissues of old rats (percents of respective parameters in young animals) [25].
1) ROS generation; 2) Diene conjugates; 3) Schiff bases; 4) Protein peroxidation; 5) Total antioxidant activity; 6) superoxide dismutase activity. Significance of differences vs. young rats: * $p<0.05$; ** $p>0.01$; *** $p<0.001$.

Effects of epithalamin and melatonin on free radical processes in rats

In a series of experiments we studied the effects of Epithalamin and melatonin on indicator parameters of free-radical processes in the blood serum of rats [12–14]. Epithalamin was administered to rats aged 2–3 months for 5 days in the morning as subcutaneous injections at the dose of 0.5 mg per animal. Melatonin was given with drinking water (20 mg/l). As seen from data presented in Table 2, Epithalamin significantly suppressed blood serum chemoluminescence (2.8-fold) and lipid peroxidation, the latter effect manifested, in particular, as a pronounced reduction of diene conjugates (4.1-fold), whereas Schiff bases revealed only a trend to decrease (by 14,4%, $p>0.05$). These observations suggest that treatment with Epithalamin affects the initial stages of LPO. Melatonin also suppressed LPO as suggested by the decrease of either diene conjugates and Schiff bases. It should be noted that treatment with melatonin resulted in an increase of carbonylated protein derivatives (by 12%, $p<0.05$), whereas Epithalamin did not influence protein peroxidation. Treatment with either Epithalamin or melatonin was associated with a significant (by 36.6%, $p<0.01$) increase of the total antioxidant activity. One week after the onset of treatment with Epithalamin, SOD activity in blood serum increased (by 19,7%; $0.05<p<0.1$), whereas treatment with melatonin suppressed the activity by 31.6%. The same trends were displayed by serum ceruloplasmin, albeit within the normal range. However, in rats treated with Epithalamin, SOD and ceruloplasmin levels in blood serum were significantly higher (by 75%, $p<0.01$, and by 27%, $p<0.05$, respectively) than in rats treated with melatonin. Treatment with melatonin was also associated with a trend to a decrease in the content of nitrites generated by oxidation of nitric oxide produced by blood vessel endothelium.

The above results confirm the data about the antioxidant effects of melatonin [10–13, 32] and Epithalamin [12, 13]. Recently, Somova et al [33] have shown that treatment with melatonin and Epithalamin decreased the contents of LPO products in rat liver (by 33% and 59%, respectively), and increased the levels of reduced glutathione in rat liver (by 28% in both cases). It should be stressed that, though either products of the pineal gland exhibit pronounced antioxidant effects, their mechanisms seem to be different. Epithalamin increases SOD and ceruloplasmin, whereas melatonin does the opposite. Probably, the antioxidant effects of melatonin, at difference from those of Epithalamin, are associated with the antioxidant activity of the former, i.e., its ability to directly react with free radicals generated in a living body from molecular oxygen and lipids, in particular hydroxyl (•OH) and peroxyl (•OOR) rad-

Table 2. Effects of Epithalamin and melatonin on parameters of free-radical processes in blood serum of rats [12,13]

Parameter	Control (n=8)	Melatonin (n=6)	Epithalamin (n=10)
Serum chemoluminescence, units	7.40 ± 0.99	4.53 ± 0.61***	2.64 ± 0.61*
Diene conjugates, nmole per mg protein	0.99 ± 0.12	0.28 ± 0.36*	0.24 ± 0.04*
Schiff bases, units per mg protein	4.87 ± 0.36	3.39 ± 0.35**	4.17 ± 0.38
Antioxidant activity, units per mg protein	0.82 ± 0.03	1.12 ± 0.03*	1.12 ± 0.04*
Carbonylated amino acid derivatives, 10^{-2} mmole per mg protein	8.58 ± 0.34	9.59 ± 0.29***	8.02 ± 0.23
SOD activity, units per mg protein	1.93 ± 0.03	1.32 ± 0.07*	2.31 ± 0.24*#
Ceruloplasmin, mg %	34.7 ± 3.9	29.0 ± 2.1	36.8 ± 3.0##
Nitrite (NO_2^-), nmole per mg protein	16.4 ± 1.7	20.8 ± 1.2	21.4 ± 2.3

Note: For each analysis sera obtained from 2–3 rats were pooled.
Significance of differences vs. control: * $p<0.001$; ** $p<0.01$; *** $p<0.05$
Significance of differences vs. melatonin-treated rats: # $p<0.01$; ## $p<0.05$

icals [9–11]. By contrast, the antioxidant effects of Epithalamin seem to be medicated by enzymic antioxidant defenses. This suggestion is in accord with data about comparable antioxidant effects of melatonin *in vivo* and *in vitro* [9–13, 32], whereas Epithalamin appears to be much more potent *in vivo* than *in vitro*. Further studies involving the determination of low molecular antioxidants (ascorbic acid and α-tocopherol) as well as thiol-containing compounds (glutathione, in the first place) are needed to elucidate differences between the mechanisms of *in vivo* antioxidant effects produced by melatonin and Epithalamin.

Many natural and synthetic antioxidants are known to possess a wide range of biological activities including, in particular, the abilities to improve some immune functions, to serve as geroprotectors, and to prevent atherosclerosis and cancer [17, 19]. Either melatonin or Epithalamin have been shown to increase animal life span, stimulate immune responses, and inhibit tumor development [2, 8, 10, 34]. At the same time, epiphysectomy or 24-hour illumination known to inhibit pineal functions decrease animal life span, suppress immune functions, and promote atherosclerosis and tumor development [1, 10, 34, 35]. With regard to the fact that Epithalamin stimulates the synthesis and secretion of melatonin whose level decreases with aging [1, 10, 34], the above effects of Epithalamin are likely to be mediated by the ability of melatonin to scavenge free radicals. However, the use of Epithalamin may appear to be more beneficial compared with that of melatonin, because the former produces not only direct antioxidant effects but, also, stimulates enzymic antioxidant systems exemplified by SOD and ceruloplasmin.

Effects of Epithalamin and melatonin on free radical processes in Drosophila melanogaster

We have studied the effects of Epithalamin on LPO in *Drosophila melanogaster*, strain VES, selected from the natural Leric population for high embryonic mortality and subject to strict inbreeding for about 300 generations [36]. The VES strain displays a unique dynamics of embryonic mortality characterized by the rise of early embryonic death rate from 65% on the first day of egg laying to 95% by the forth day [37]. One possible cause of such dynamics is the rapid senescence of females. The association of this phenomenon with LPO is widely discussed in literature.

Epithalamin and melatonin were diluted to 0.01% in ethanol and added to growth media at a 0.01% concentration. This media was used to treat larvae aged 2–3 days, at which age the effects are produced, which are the most significant in influencing the adult life span. LPO products were determined in 11-days old flies.

The analyses of LPO in flies of different groups (Table 3) have shown that control flies exhibit a marked sexual dimorphism, i.e., the levels of diene conjugate-containing hydroperoxides and ketones are significantly lower in males than in females (by 40% and 49%, respectively), which is inverse to differences between their life spans. Treating flies with Epithalamin resulted in a significant decrease of diene conjugate-containing hydroperoxides and ketones (2.3-fold and 3.4-fold, respectively, $p<0.001$) eliminating sex-specific differences in these LPO indicators. This effect is likely to contribute to the geroprotector activ-

Table 3. Effects of Epithalamin and melatonin on peroxidation in D. melanogaster [8]

Parameter	Control	Melatonin	Epithalamin
	Females		
Diene conjugate-containing hydroperoxides (nmole/mg)	0.976 ± 0.079	0.462 ± 0.137**	0.416 ± 0.079**
Diene conjugate-containing ketones (nmole/mg)	0.519 ± 0.044	0.181 ± 0.075**	0.152 ± 0.044*
SOD activity (units per mg protein)	135.8 ± 11.24	114.0 ± 12.14	146.8 ± 13.30
Catalase activity (mmole H_2O_2·min^{-1}·mg $protein^{-1}$)	41.3 ± 1.46	51.4 ± 1.58**	49.4 ± 1.76**
	Males		
Diene conjugate-containing hydroperoxides (nmole/mg)	0.584 ± 0.097*	0.426 ± 0.097	0.304 ± 0.079***
Diene conjugate-containing ketones (nmole/mg)	0.260 ± 0.053*	0.154 ± 0.053	0.138 ± 0.044
SOD activity (units per mg protein)	134.1 ± 13.30	109.6 ± 14.87	189.4 ± 14.87***
Catalase activity (mmole H_2O_2· min^{1}·mg $protein^{-1}$)	89.3 ± 1.94*	87.8 ± 1.94*	95.5 ± 1.94***

The difference with corresponding parameter in females is significant, *P<0.001
The difference with sex-matched controls is significatn, **P<0-01; ***P<005.

ity of Epithalamin in fruit flies [7, 36]. Inhibition of LPO by melatonin was less pronounced compared with that of Epithalamin. Melatonin did not change catalase activity in males but increased it in females (by 24%, $p<0.02$) and did not change superoxide dismutase in both sexes. Epithalamin significantly increased catalase in males and females and increased SOD in males by 41% (Table 3).

The data obtained with mice and fruit flies provide the evidence that Epithalamin and melatonin are antioxidants. We have found that Epithalamin increases melatonin synthesis in old rat pineal gland and its secretion into the circulation [2]. Similarly to melatonin, Epithalamin inhibits LPO and increases catalase activity in mice and fruit flies. However, in contrast to melatonin, Epithalamin increases serum SOD activity and ceruloplasmin in rats and SOD activity in fruit flies [7, 12]. It has been shown that the lifespan of transgenic *D. melanogaster* is increased only when the doses of either genes, SOD and catalase, are increased, but not when the dose of any of these genes is increased separately [38]. Recent studies have demonstrated that transgenic *Drosophila* fruit flies with the increased expression of SOD1 gene in their motoneurons live 40% longer and are much more resistant to the oxidative stress compared with the parent flies [39]. Moreover, the expression of SOD, catalase, glutathione peroxidase and xanthine dehydrogenase has been demonstrated to be significantly higher in long-lived *D. melanogaster* strains than in short-lived ones [40].

On the whole, the results of our *in vitro* and *in vivo* experiments suggest that Epithalamin possesses a clear antioxidant activity, which can contribute to geroprotector effects of Epithalamin. This activity can partly be mediated by stimulation of melatonin synthesis and secretion and partly, by the ability of Epithalamin to stimulate the expression of SOD and other antioxidant enzymes.

Effects of Epithalamin on free-radical processes in humans

The above experimental data justified studies of the antioxidant effects of Epithalamin in clinical settings. In 1991 the Pharmacological Committee of RF Ministry of Public Health licensed Epithalamin for clinical use. No data about melatonin effects on free-radical processes in humans are available. In many countries, including Russia, melatonin is not included in Pharmacopoeias and is licensed only as a food additive.

The antioxidant activity of Epithalamin was studied in a group consisting of 56 patients of different ages (20–45 years: 26 patients; 46–59 years: 15 patients; 60–74 years: 15 patients). Blood serum antioxidant activity [41, 42], LPO product contents, and superoxide dismutase and glutathione peroxidase activities were determined before and after treatment with Epithalamin, which consisted of intramuscular injections of 10 mg of the preparations performed daily for ten days.

The age-dependent decrease of the total antioxidant and anti-radical activity of serum was found in human subjects studied (Table 4), suggesting a disturbed balance of pro-oxidant and antioxidant systems. The method that we used to evaluate the total antioxidant activity of serum is based on registering the decrease of chemo-luminescence induced by the reaction of riboflavin radicals with Fe(II) [41]. It is believed that riboflavin radicals are scavenged by serum proteins and low molecular components [41, 43]. The anti-radical activity of serum was assessed in the protein-free supernatant reacting with a stable radical 1,1-diphenyl-2-picryl-hydrazyl, the reaction being influenced by the presence of low molecular antioxidants, such as ascorbic acid, α-tocopherol, glutathione, and, to a lesser extent, by retinol, uric acid,

and unidentified compounds. Our data conform to observations of other authors who showed that the reduction of the antioxidant activity and other indicators of anti-radical defenses occur in aging humans [21, 26]. The increase of peroxidative damage to aging human tissues is evidenced by data demonstrating that the levels of 8-OH-2-deoxyguanosine, a marker of oxidative damage to the mitochondrial DNA, and of malonic dialdehyde and carbonylated proteins increase in human muscles over the period ranging from 25 to 93 years [44].

In patients suffering from age-dependent pathological conditions we observed a significant increase of LPO products vs. the respective values found in healthy donors. Treating the patients with Epithalamin resulted in a significant increase of the total antioxidant and anti-radical activities, decrease of LPO products, and increase of superoxide dismutase and glutathione peroxidase activities in blood serum (Table 5). These data are in accord with the above results obtained with experimental animals.

Our clinical results provide the evidence that the use of Epithalamin is beneficial for the correction of anti-radical defenses in premature aging and age-dependent pathological conditions. Data have been obtained earlier that Epithalamin produces beneficial objective and subjective effects when used for treating women with myocardial dystrophy of vegetative and dyshormonal origin [4, 45]. Epithalamin appeared to ameliorate disturbed immune balance, primarily by affecting cellular immunity, and to restore hormonal balance in these women. The use of Epithalamin for prevention and treatment of age-related pathological conditions in two groups of aged human subjects (60–95 years) have revealed its high homeostatic activity [5], which was evaluated using the coefficient of homeostatic stability, i.e., the proportion of the number of biochemical, immune and endocrine parameters found to be within ranges assumed to be normal for young adults (25–45 years) to the number of parameters outside such ranges [46] (Fig. 2). Studies conducted at The Research Institute of Gerontology of The Ukrainian Academy of Medical Sciences

Table 4. Parameters of blood serum antioxidant defenses in human subjects of different age-groups

Age-group	Number of subjects	Total antioxidant activity (units/ml)	Total anti-radical activity (μmole/l)
I (20–45 years)	26	82.8 ± 1.6	950.3 ± 34.6
II (46–59 years)	15	76.7 ± 2.6*	727.4 ± 28.4*
III (60–74 years)	15	68.4 ± 1.5**	517.6 ± 29.5**

* The difference from Group I is significant ($p<0.05$)
** The difference from Group II is significant ($p<0.05$)

Table 5. Changes in parameters of antioxidant defenses of blood serum in humans treated with Epithalamin for age-dependent pathological conditions

Parameter	Normal value #	Before treatment	After treatment
Total antioxidant activity (units/ml)	91.8 ± 2.3	72.1 ± 3.5*	83.9 ± 2.7**
Anti-radical activity (μmole/l)	932.8 ± 36.4	676.3 ± 64.3*	808.2 ± 31.3**
LPO products (OD units/ml):			
Diene conjugates	0.59 ± 0.08	0.82 ± 0.06*	0.87 ± 0.09
Ketodienes	0.06 ± 0.02	0.21 ± 0.03*	0.16 ± 0.005**
Schiff bases	0.023 ± 0.004	0.12 ± 0.007*	0.05 ± 0.003**
Superoxide dismutase activity (units · ml^{-1} · min^{-1})	21.8 ± 0.8	19.9 ± 1.2	25.2 ± 1.9**
Glutathione peroxidase activity (μmole GSH · g Hb^{-1} · min^{-1})	150.1 ± 18.4	146.5 ± 11.9	201.6 ± 12.7*

Mean values determined in healthy donors
* The difference from the respective normal value is significant ($p<0.05$)
** The difference from the respective value before treatment is significant ($p<0.05$

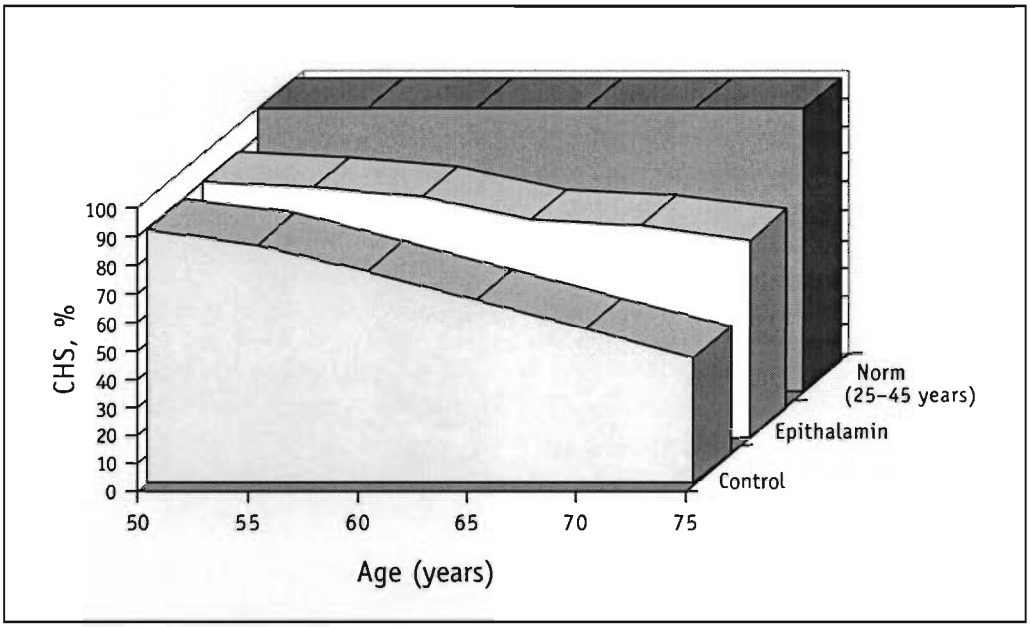

Fig. 2. Homeostatic activity of Epithalamin in middle-aged and elderly subjects [45]. CHS: Coefficient of Homeostatic Stability = (total number of normal parameters/total number of parameters) × 100.

Table 6. Effects of Epithalamin on parameters of longevity and mortality in different experimental animals [6, 8, 49, 50].

Treatment	Number of animals	Mean lifespan (days)	Time (days) of reaching a defined cumulative mortality level			Rate of aging ($\alpha \cdot 10^3 \cdot day^{-1}$)	Mean period of mortality rate doubling ($\ln 2/\alpha$)
			Medial	90%	100%		
				Rats			
Control	75	681 ± 14.5	705	825	1054	7.9 ± 0.5	5.5
Epithalamin	33	852 ± 33.8[b]	873	1050	1112	3.8 ± 0.8	6.3
Difference, %		+ 25	+ 24	+ 27	+ 6	- 52	+ 15
				Mice C3H/Sn			
Control	21	487 ± 29.4	511	691	776	7.0 ± 1.5	5.7
Epithalamin	32	640 ± 33.1[a]	679	757	885	5.1 ± 1.3	6.0
Difference, %		+ 31	+ 33	+ 10	+ 14	- 27	+ 5
				Drosophila melanogaster			
Control	199	25 ± 1.2	23	54	80	70 ± 12.9	3.7
Epithalamin	207	29 ± 1.2[a]	29	60	91	33 ± 3.3[a]	4.9
Difference, %		+ 16	+ 26	+ 11	+ 14	- 53	+ 32
				Caneorhabditis elegans			
Control	12	17.4 ± 2.5	18	-	29	-	-
Epithalamin	12	19.6 ± 2.2	19	-	29	-	-
Difference, %		+13%	+6%		0 %		

The significance of difference vs. control: * p<0.01; ** p<0.001

have also demonstrated that Epithalamin is efficient in treating premature aging [47, 48]. In experiments using four animal species (rats, mice, fruit flies, and nematodes) we have found that Epithalamin is a potent geroprotector (Table 6) [2, 6–8, 49]. In studies using rats and fruit flies the geroprotector effect of the preparation has been found to correlate with its antioxidant activity [8]. The significant increase of indicator parameters of the antioxidant defenses in blood serum of Epithalamin-treated patients is another argument in favor of the clinical use of Epithalamin as a geroprotector drug.

It is generally known that ionizing radiation is a potent inductor of free-radical processes, whereas antioxidants offer radioprotection, e.g., reduce the rate of radiation-induced mutations in peripheral blood lymphocytes [19, 51, 52]. In our experiments, Epithalamin significantly inhibited carcinogenesis induced by whole-body X-ray irradiation and chemical carcinogenesis [2]. Administration of Epithalamin to oncological patients subject to radiotherapy ameliorated the deterioration of their immune parameters [4, 45]. Thus there are reasons to believe that the antioxidant activity of Epithalamin can significantly contribute not only to its geroprotector effects but, also, to its ability to protect against adverse effects of radiation and chemical carcinogens.

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