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Relation of antistress and geroprotective effects of deuterium depleted water in aging female rats

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Abstract

The use of deuterium depleted water (DDW) in a dosing regimen of 25–30 ml per day for five weeks in aging female rats (20–22 months) contributed to the overall correction of age-related disorders of the adaptive status, estrous cycle regulation and nonspecific resistance, as well as the appearance of visually distinct signs of rejuvenation of the animals. The obtained results show the relation between the geroprotective effect of DDW and the development of the general nonspecific antistress adaptation reactions of calm and elevated activation that can serve as an experimental basis for the development of an effective method of geriatrics and prevention of cardiovascular diseases and degenerative processes in women in the peri- and postmenopausal period.

Keywords

Deuterium depleted water, Aging female rats, Estrous cycle, General nonspecific antistress adaptation reactions, Neuroendocrine regulation

Imprint

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Introduction

Age-linked disorders of the neuroendocrine and immune regulation, as a rule, lead to a reduction in the nonspecific resistance of an organism and are responsible for development of various pathological states, which may include cardiovascular, degenerative and tumor-related diseases [1, 11, 21]. In addition, there are some specific sex-linked differences in the age-associated disorders, which manifest themselves as a faster decline in the reproductive function in the female organism in comparison to the male one, along with the existence of a certain relation between the hormonal function of the ovaries and the resistance of the female organism to cardiovascular and some other diseases [19, 28]. It has been demonstrated that, at the level of the entire organism, the key role in providing aging-retarding conditions should be assigned to a mechanism responsible for generation and changes of some discrete states in the organism, namely, some integral adaptation reactions of stress [20] and antistress [3, 4] in character. An accumulation and predominance of aging-related stress alterations provoke a gradual decline in the nonspecific resistance. In contrast, the development of stable reactions of the antistress type contributes to activation of CNS, the endocrine and immune systems, the energetic and peroxidation metabolism, i.e. the most important components governing an elevation of the organism resistance to unfavorable external and internal environment factors.

Progression of age-associated disorders depend on many of exogenous and endogenous factors, among them the peculiarities of the water condition profile in an organism that is of vital importance for biochemical processes and realization of effects produced by weak information actions of water due to the unique cluster structure of water and its metastability [18, 26]. In this case, it should be mentioned that known are some biotropic properties of water, which are determined by its actual isotope composition. It has been detected that even some slight changes in the relationship between the stable hydrogen isotope ratios may have a noticeable effect on the states of a living organism. By this means it has been found that water enriched with the deuterium is quite often the cause of some pathological changes in the organism; in case when we deal with a shift in the water isotopic composition to light protium water, on the contrary, this type of water is capable of improving the functional state of a human or an animal [24, 25, 27]. A number of favorable effects produced by *deuterium depleted water* (DDW) on the conditions of various protection systems in the organism have been detected [7, 17, 23], up to and including pronounced triggering action by DDW on the mechanisms of the anti-tumor resistance [16, 22]. Evidence data obtained from epidemiological studies show that there are cases of extreme longevity for those individuals reported who drink original water supplied by some natural springs, differing from standard drinking water in a number of its properties, among which is deuterium depletion [15]. All this allows us to consider DDW use as a new way for correction of some homeostatic disorders associated with aging.

Our challenge was to carry out experimental studies addressing a corrective effect produced by DDW on the adaptation status, the state of the estrous cycle and the microflora composition on skin surfaces in outbred female Albino rats in their aging period.

Materials and methods

Our experimental studies were performed in the early spring period in 27 outbred Albino aging female rats aged 20–22 months, who had weights 290,7 ± 31,6 g, against 10 young female rates aged 8–10 months with a weight of 210,4 ± 31,6 g. All our experimental studies involving use of animals have been carried out in full compliance with the applicable international bioethics rules, guidelines and regulations [12]. The aging animals were divided into two groups: Group A and Group B, correspondingly. Group A included 13 rats who received DDW with a daily amount of 25–30 ml during 5 consecutive weeks. Group B covered 15 female rats who received only standard drinking water, the quality of which met the requirements specified by the relevant Russian National Standard GOST. DDW had a deuterium concentration three times lower than it was the case with regional standard-type natural water (150 ± 6 ppm) and showed no essential differences from the latter in its mineral analysis.

In order to assess effects induced by DDW on the adaptation status of the animals, in our experimental studies traced was dynamics of progression of general nonspecific adaptation reactions (GNARs) of the organism [3. 4]. Each type of GNARs, covering the reactions of acute stress and chronic stress as well as the antistress reactions of training, calm and elevated activation, was identified according to the Shilling's blood count data with the use of Giemsa – Romanovsky staining technique, with a 200 blood cell counting accuracy. In doing so, the percentage of lymphocytes in peripheral blood was taken as an identifier making possible to properly attribute each actually initiated reaction to the respective GNAR type. The corresponding leukogram patterns in the animals were identified first initially, prior to the experimental studies, and later, on a regular weekly basis, in the course of the experiment. Blood samples were taken from a rodent femoral vein located under the skin in the cellular tissue, throughout the medial surface, in the morning time before feeding. For the purpose of processing of the experimental data, utilized was our original Software Statistica 6.0, and the statistical analyses were completed in accordance with Student's t-test technique.

Cytological data on the functional status of the ovaries were obtained using vaginal smear reading. Upon flushing from the vaginal lining, smears were read with the Leica DM LS2 microscope two times a day, in the morning and in the evening, respectively. For that purpose, the following phases of the estrous cycle were traced: 1. Diestrus (D) phase which implies the functional rest and which is marked by the presence of leu-kocytes and mucus found in the smears; 2. Proestrus (P) phase recognized by the presence of polygonal, usu-ally nucleated, epithelial cells, with excentrically located nuclei; 3. Estrus phase (O) when non-nucleated cornified squamous epithelial cells appear and form large clumps and sheets; 4. Metestrus phase (M), the day after estrus, exhibiting a mixed cell composition, when a mixture of cornified squamous epithelial cells and leukocytes predominates. Length of each phase or stage and their sequences within the estrous cycle were recognized that has made possible to evaluate periodicity of hormonal changes in the ovaries [2].

In order to assess the state of the nonspecific resistance of the organism, under administering DDW to aging female rats, applied has been the trivial assessment procedure by Klemparskaya – Shalnova for the proper identification of autoflora species on the skin as current status testing for information [6]. To examine the actual nomenclature of the microorganisms sampled from rat tails with the agar replica test technique, the Korostylev medium consisting of meat-peptone agar with an addition of a 1,5 % Bromthymolblau alcohol solution and a 1 % mannit solution was used. The cultivated bacteria have been differentiated and recognized according to their ability to ferment mannit and change color of the colonies of Staphylococci both of pathogenic and non-pathogenic nature.

Results and discussion

Our analysis of leukocyte count data on peripheral blood in aging rates in the above mentioned Groups completed first before our experimental studies has revealed that the animals in 88,6 % of cases had low relative numbers of lymphocytes (30–45 %) at a rather high total amount of leukocytes in blood (see Table 1 below herein). Some cases of aneosinophilia were reported, too.

The said indices were in correspondence to the leukogram pattern typical for the symptom complex of stress. Moreover, they were statistically significantly different from the hematological characteristics in the female rats at a young age, whose lymphocyte percentage was 1.7 times greater as compared with the aging rats, with a lower total leukocyte count and with no cases of aneosinophilia.

Upon completion of the DDW treatment course, the animals in Group A demonstrated the normalized leukogram pattern. First it was applicable to the relative number of lymphocytes as the key index, showing the type of the adaptation reaction of the organism, which was higher by a factor of 1,5 in comparison with the initial state (see Table 1 below herein). The total count of leukocytes in blood became normal, too, and no aneosinophilia cases were observed. The peripheral blood leukogram changes in question made it possible to recognize the development of the integral reactions of the physiological classes in the Group A rats as follows: GNARs of training, calm and elevated activation. The hematological data obtained from the animals in Group B, who received standard drinking water, practically did not show any differences from their initial values: an elevated total count of leukocytes was recorded, and lower lymphocyte percentages were reported than those identified in the young animals. So, an analysis of developing GNARs of differing types within each Group and between the specified Groups has shown that the use of DDW instead of the standard drinking water has resulted in a substantial increase in the occurrence rate of the antistress reactions detected in Group A covering the aging female rats.

Table 2 below herein summarizes variability in the occurrence rate of different GNARs for a five week period in aging female rats of the Groups under examination. As may be seen from the Table, the occurrence rate of the stress reaction in Group A identified prior to the administration of DDW was comparable with that reported for Group B, and the occurrence rate of the antistress reactions of different types has varied. Upon DDW receiving, during the first week, reported were GNARs of the antistress types in the Group A rats only, and the same stable maintenance of the physiological reactions was recorded till the completion of the experimental studies. In the animals of Group B, in 35% of cases, in contrast thereto, observed was stabilization of the development of the stress reaction pattern, which was especially apparent by the end of the experiment. Differences of decisive importance between the aging animals belonging

Table 1. Leukogram data as percentages of major blood cells in aging female rats under DDW influence as against their initial state and as against the state of the reference group of young and aging female rats without DDW administration

Blood cell types	Young female rats	Aging female rats			
		Initial state	After DDW administration		Reference
			Group A: activation	Group A: training	Group B: stress
Total leukocyte count /mcL	7300±1200	17100±2900 [1]	9100±1700 [2]	10800±1200 [2]	16500±1700 [1]
Lymphocytes, %	75,5±4,5	44,0±2,94 [1]	68,0±2,45 [2]	56,5±1,7 [2]	37,5±2,15 [1]
Eosinophils, %	1,0±0,13	0,5±0,11	1,0±0,7	1,5±0,1	0
Neutrophils, %	27,5±3,5	42,0±2,7 [1]	30,0±3,3 [2]	46,0±2,9	62,0±4,4 [1]

Notes:

[1] statistically significantly differing from the values in young female rats (p<0,001);

[2] significantly differing from the values in aging female rats in their initial state (p<0,001).

Table 2. Relations of occurrence rates of different adaptation reactions in aging female rats receiving DDW (Grpup A) vs. those receiving standard drinking water (Group B)

Types of adaptation reactions	Relation of occurrence rates of ARs of the same name (%) in aging animal groups under examination (A vs. B)					
	Initially	Week 1	Week 2	Week 3	Week 4	Week 5
Stress	20,3/21,4	0/21,4	0/21,4	0/35,7	0/35,7	0/35,7
Training	44,1/40,8	30,8/31,4	30,8/57,2	15,4/50,0	15,2/50,0	0/50,0
Calm activation	20,6/20,6	38,4/28,6	23,1/21,4	38,4/14,3	30,8/14,3	30,8/0
Elevated activation	15,0/17,8	30,8/18,6	46,1/0	46,2/0	54,0/0	69,2/14,3
Intra-group relation between ARs: Antistress / Stress reation in Group A	3,9	AS only	AS only	AS only	AS only	AS only
Intra-group relation between ARs: Antistress / Stress reation in Group B	3,7	3,7	3,7	1,8	1,8	1,8

to different Groups were also found in the occurrence rates of the reactions of the same name of the antistress types. So, beginning with week 2 till week 5 of the experiment time, much more frequently (up to 3–7 times) observed were the reactions of the calm and elevated activation in Group A, which were considered to be the most favorable for the neuroendocrine regulation, against those seen in Group B, where and when the reaction of stress was accompanied by the antistress reaction of training.

The established inter- and intra-group relations between the antistress (AS) and stress (S) reactions have demonstrated the dominance of the antistress link within the entire structure of the adaptation reactions in the animals, who received DDW, and actually it has shown high effectiveness of the DDW action. As is known, the calm activation GNAR and the elevated activation GNAR are featured by higher percentages of lymphocytes in blood (for outbred rats 61–70 % and 71–80 %, correspondingly), in comparison with the stress GNAR and that of training (under 50% and under 51–60 %, correspondingly). The above performed correlation & regression analysis of the relationship between the level of lymphocytes and the respective morphological, biochemical and hormonal indices of the functional state of the nervous, endocrine and immune systems in the organism has revealed that we deal with high values of the paired and multiple correlation coefficients as well the determination, which are decisive for production of a strong correlation between the key index and the multisystem attributes of an adaptation reaction [9, 10].

As is seen, the mechanism of development of the antistress calm and elevated activation GNARs has contributed to a favorable correction of the length of the estrous cycle stages in the rodents. The final data obtained in the Groups in question by the end of the experimental studies should be treated as evidence in support of the DDW effect on the organism for the aging female rates in Group A. As is indicated in Table 3, the estrous cycle length in the aging animals in Group B, who received standard drinking water, differed markedly from that recorded in the young animals in the reproduction age and was featured by a considerably reduced length of the proestrus and estrus stages (2-3 times) with a significantly increased length both of the metestrus phase (more than 3 times) and dioestrus (more than 1.5 times) stage. The DDW use for five consecutive weeks resulted in the statistically significant changes found in the estrous cycle that should be considered as evidence for the partial restoration of the reproductive function in the aging female rats in Group A. So, by the end of the experiment, the animals in Group A have demonstrated a substantial shortening of the length of the metestrus and dioestrus stages (1,5 and 1,8 times, respectively) and a lengthening of the proestrus stage, as compared with Group B, that has placed the said indices closer to those recorded for the young age rats (see Table 3 below herein).

When comparing the length of the individual stages in common within the estrous cycle over the entire period of observations, under developing of GNARs of various types, a clear-cut relationship between the integral changes and the hormonal fluctuations was also traced. It appeared as the restored length and sequence of the individual stages within the cycle that was primarily detected under formation of the stable reactions of activation under the DDW effect. As evidenced by the data given in Table 4, when supporting GNARs of the calm and elevated activation in the Group A rats, the length of each individual

Table 3. DDW administration effect on the length of individual stages of estrous cycle in aging female rats

Animal Groups		Length of a separate stage of the estrous cycle (hours)			
		D	P	O	M
Norms for young age		57 (55–57)	12 (12–14)	27 (25–27)	6 (6–8)
Aging female rats	Group B	91,1±7,0	4,1±0,7	15,0±2,4	25,5±3,8
	Group A	51,2±5,4 [1]	9,1±1,8 [1]	13,8±2,7	16,5±2,4 [2]

Note:
[1] statistically significant differences from values in Group B, p < 0,01–0,05

stage of the cycle in common, considering a period of 30-35 days, was brought closer to the normal indicative values, that is to say, to those values identified in the young rat Group. So, the length of the diestrus stage in general in those cases decreased approximately by 50 hours in comparison to the same value in Group B, and it was found to be maximally close to the values detected in the young age animals. The duration of the proestrous stage in the Group A rats, with the development of GNAR of the calm and elevated activation, demonstrated no difference from the normal values. At the same time, in the animals in Group B, with the prevalence of GNAR of training, when and where the reaction of stress was often recorded, the length of the above mentioned stage was 3 times shorter than that reported for the young female rats.

The restoration of the estrus stage, which either has been absent in the initial state or exceeded by 1.6 times the limits of the stage range in young female rats, is of considerable importance for an evaluation of the geroprotective effect produced by DDW with the development of GNAR of calm and elevated activation. The duration of the metestrus stage in female rats in group A exceeded the norm, but was recorded to be statistically significantly shorter than that under the development of the training reaction. In cases of development of the reaction of the same name in animals in group B, the process of the estrous cycle normalization was moderately expressed only, but the indices of change of the phases and their length in common differed from those recorded under stress, which was characterized by profound disorders in the sexual function rhythmic activity in aging female rats in the reference group under examination.

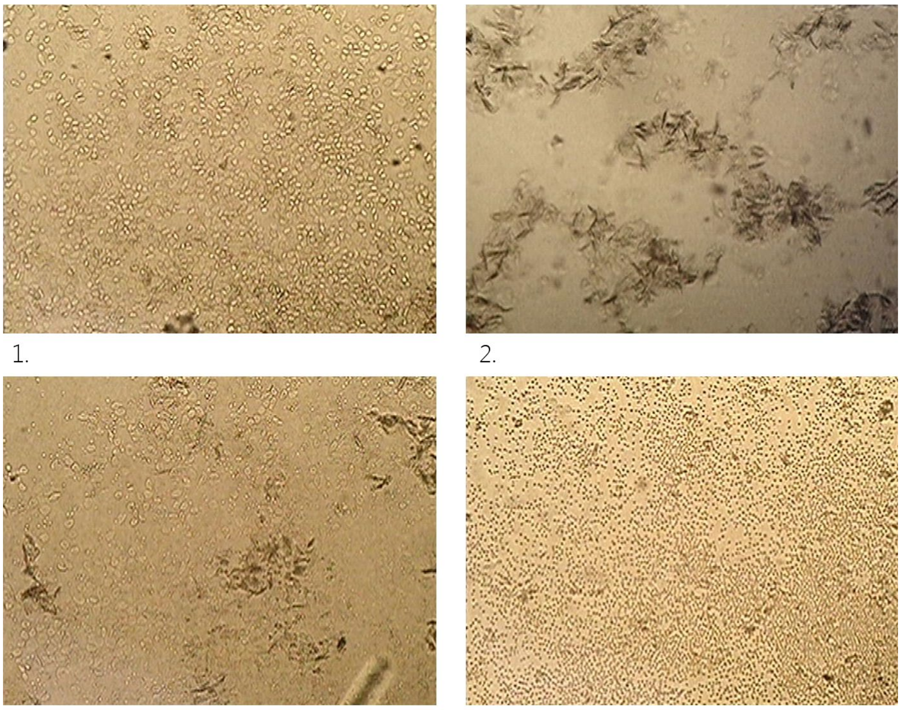
Microscopic examination of vaginal smears in the aging female rats provided a means for visualizing the regulatory effect by DDW at the cell level. Figure 1 below herein exhibits vaginal smear readings obtained in the aging female rats who received DDW, where the cell composition corresponds to the normal cell types of the estrous cycle stages. Epithelial cells prevailed in the proestrus phase; squamous epithelial cells dominated in the estrus phase; mixed composition of cells (cornified squamous cells, epithelial cells, leukocytes) were dominant in the metestrus phase, and leucocytes were prevalent in the diestrus stage.

An interpretation of the obtained results from the standpoint of the estrous cycle neuroendocrine regulation

Table 4. Change in length of individual stages within the estrous cycle in general during the experiment period (in hours) in aging female rats with the development of different GNARs under DDW effect

Stages of estrous cycle	Young female rats	Aging female rats		
		Group B	Group A	
		GNAR of training and stress	GNAR of calm and elevated activation	GNAR of
Diestrus	346,6±7,2	389,4±42,2	training	316,8±19,9
Proestrus	71±2,4	24,1±1,7 [1]	78,1±2,4 [2]	60,3±7,9 [1,2]
Estrus	148±14,7	235,1±39,1 [1]	144,7±11,3 [2]	64,5±7,6 [1,2]
Metestrus	32,4±2,2	20,5±3,8 [1]	47,4±2,3 [1,2]	58,4±2,7 [1,2]

Notes:
[1] statistically significantly differing from values in young rats (p<0,01)
[2] statistically significantly differing from values in aging rats (p<0,01)



1. 2. 3. 4.

Figure 1. Cell composition of vaginal smears read at different stages of estrus cycle in aging female rats under DDW influence:
1) proestrus; 2) estrus; 3) metestrus; 4) diestrus.

in the context of the dominant integral reaction of activation, under the DDW effect, has demonstrated that the stimulation by estrogens, which are produced by ovaries and initiate the vaginal proliferative activity, determines also the cell composition. An indication thereof firstly are the implied DDW effects responsible for approaching the normal value of the proestrus stage, that indirectly confirms the fact of the activation of hypophysis follicle-stimulating hormone secretion. Secondly, despite the fact of the normalization of the estrus period, it has become apparent that we deal with a decrease in the metestrus period length with reference to the respective values in the aging female rats as an evidence of the corpus luteum shrink and inhibition of excessive luteinizing hormone secretion. And, thirdly, the noted normalization of the metestrus stage has shown there has been found the state of hormonal balance approaching.

The studies identifying the skin autoflora peculiarities in aging female rats have also revealed some differences attributed to the type of evolving GNARs by comparing two cases: that with DDW administration and another one with use of standard drinking water (see Table 5 below herein).

Thus, with the development of the stable calm and elevated activation reactions, under the DDW effect, observed has been a noticeable suppression of growth up to even complete disappearance of pathogenic staphylococcus with a negative mannit effect of the "yellow" colonies formation, while the "green" colonies with a positive mannit effect of nonpathogenic forms of staphylococcus remained. With the development of the training reaction, the "green" colonies significantly prevailed over the "yellow" ones. Under stress, in animals of Group B observed has been an increased pathogenicity in skin autoflora: against the background of suppression of the "green" colonies, a progressive growth in the «yellow» colonies of pathogenic staphylococcus has been detected. Hence, the dominant in Group A adaptation reactions of calm and elevated activation are the most efficient, when considering the antimicrobial resistance.

The antistress effect induced by the DDW administration led to an appearance of visually distinguishable signs of rejuvenation in the Group A animals. In the female rats, receiving DDW, a change in hair coat was recorded. Observed was replacement of a coarse stiff hair coat, yellowish toned, comprising multiple local areas characterized by varying levels of hair loss, by a white soft fur with a new thick undercoat; the eye sclera have brightened (see Figure 2), and the motion activity significantly higher in the animals under examination was also reported.

Thus, the obtained data demonstrate pronounced geroprotective DDW effect associated with DDW antistress action which has manifested in formation of stable GNAR of the calm and elevated activation, contributing to a considerable increase in nonspecific resistance and initiation of the restoration process in the aging female rats. It is obvious that the said effects take place due to such a specific property of DDW as its capability to activate the cell metabolism and elevate excitability of the neuroendocrine regulation centers [13, 14].

The data on the development of GNAR of the calm and increased activation under the DDW influence are in good agreement with the known findings in favor of the activating effect

Table 5. Changes in skin autoflora in aging female rats under DDW effect, depending on the adaptation reaction type

Types of microflora colonies	Group B GNAR of stress	Group A	
		GNAR of calm and elevated activation	GNAR of training
Total number of the colonies	43,6±2,18	57,0±1,6 [1]	34,3±1,27 [1]
"Yellow" colonies – pathogenic staphylococcus	36,9±5,1	N/A	5,9±1,3 [1]
«Green» colonies – nonpathogenic staphylococcus	7,0±1,3	54,5±3,05 [1]	26,3±2,8 [1]

Note:
[1] statistically significantly differing from values in group B, p<0,01

1.

2.

Figure 2. Change in appearance of aging female rats 5 weeks after DDW administration.
1. Group B: Female rats receiving drinking water.
2. Group A: Female rats receiving DDW.

made by DDW on the CNS serotonergic structures [25], since the relationship between the given GNAR development and an increase in serotonin concentration in brain structures and blood formed elements had been established earlier [5]. This fact is of great significance due to an important role of stress-limiting serotonergic processes for the purpose of ischemic heart damage prevention and a reduction of cardiovascular disease risks [7] related to pathologies associated with aging.

The evidence of the correlation between mortality due to cardiovascular and degenerative diseases and a decline in the reproductive function in females during peri- and postmenopause reveal an additional aspect in the important role of properly maintaining the ovarian hormonal function to provide resistance of the female organism to damaging factors [28]. In this connection, the antistress and geroprotective DDW effects detected and traced in our studies is a matter of great interest.

Conclusions

The results obtained in our experimental studies bear witness that there is pronounced geroprotective effect produced by DDW in outbred aging female rats and that there exists the relation between this effect and the development of the general nonspecific antistress adaptation reactions of calm and elevated activation. The obtained evidence data provide unique insight into the effect exerted by DDW on mammalian organisms, and it may serve as an experimental foundation for development of an effective method suitable for use in geriatrics and prevention of cardiovascular diseases and degenerative processes in females during peri- and postmenopause.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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