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Comparative Analysis of Lipid Peroxidation System in Humans and Rats with Arterial Hypertension

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Abstract

The aim of this research was to study the concentration of lipid peroxidation (LPO) products and the activity of antioxidant defense (AOD) parameters in ISIAH rats in comparison with a group of adolescents with arterial hypertension (AH).

***Material and Methods*:** We conducted the study on young (2.5-3 months) sexually mature male rats of the normotensive line (WAG) (n=20) (intact animals) and hypertensive line (ISIAH) (n=20), weighing 200-220 g. The data of 65 adolescents aged 13-17 years with AH (Group 1) were used for the clinical study (the age of adolescents is comparable to the biological age of the experimental animals used). The comparison group consisted of 65 normotensive adolescents of the same age and sex ("copy-pair" type) (Group 2). The plasma level of antioxidant parameters (total antioxidant activity [TTA], SOD activity, α-tocopherol and retinol) and primary/secondary products of LPO (conjugated dienes [CD], ketodienes and conjugated trienes [KD-CT], and thiobarbituric acid reactants [TBARs]) were determined using spectrophotometric and fluorometric methods.

Results: We found that the course of LPO reactions in animals and humans was similar, which was expressed by the activation of prooxidant factors and the insufficiency of antioxidant response. Species differences concerned the intensity and number of parameters involved in the pathological process. Thus, in ISIAH rats there was an increase in toxic TBA-active products and a decrease in TTA, α-tocopherol and retinol in comparison with normotensive animals; in AH adolescents there was an increase in the content of intermediate–KD-CT and final TBA-active products, and a decrease in the α-tocopherol level in relation to the comparison group.

Conclusion: Features of response of the LPO nonspecific system in hypertensive rats and humans allow using this line of rats for further study of adaptive mechanisms, and to extrapolate the received experimental data on humans, taking into account certain specific distinctions.**(International Journal of Biomedicine. 2019;9(4):292-296.)**

Key Words: arterial hypertension • rats, adolescents • lipid peroxidation • antioxidant defense

Abbreviations

AH, arterial hypertension; **AOD**, antioxidant defense; **BP**, blood pressure; **CDs**, conjugated dienes; **DB**, substrates with unsaturated double bonds; **FR**, free radicals; **ISIAH**, inherited stress-induced arterial hypertension; **KD-CT**, ketodienes and conjugated trienes; **LPO**, lipid peroxidation; **OS,** oxidative stress; **SOD,** superoxide dismutase; **TAA,** total antioxidant activity; **TBARs**, thiobarbituric acid reactants; **WAG**, Wistar Albino Glaxo.

Introduction

Arterial hypertension (AH) is one of the most common diseases of the cardiovascular system, characterized by a persistent increase in blood pressure.[1,2] AH often manifests itself in childhood and adolescence.[3,4]

Like any pathological condition, AH, in addition to pathological reactions, is accompanied by the inclusion of sanogenetic mechanisms reflecting the dynamic complex of defense and adaptive reactions arising under the influence of a pathogenic factor and aimed at restoring the disturbed self-regulation of the body.[5,6] Sanogenetic reactions at the cellular level consist in plastic rearrangements of cell membranes, intracellular structures, macromolecules and their medium.[7] In this case, the main role belongs to the LPO-AOD reactions, an important regulatory mechanism involved in maintaining cell homeostasis.[8-10] Its predominant role in modification of cell membrane structure, xenobiotic metabolism, immune response regulation, cell proliferation, vascular permeability, and receptor sensitivity is well known.[11] Currently, some poorly studied sanogenetic mechanisms of hypertension, in particular, the role of nonspecific reactivity of bodily reactions in the genesis of the disease, are also recognized. The lack of clear ideas causes greater interest among researchers in modeling this pathological condition, finding ways to correct it and developing new treatment methods.[12,13] At the same time, scientists know about different reactivity and types of adaptation strategies of animals and humans in response to the influence of disturbing factors.[14-16] In the context of the above, it is extremely interesting to compare the systems that indicate the development of OS in humans (adolescents with hypertension) and in animals with hereditary hypertension.

The aim of this research was to study the concentration of LPO products and the activity of AOD parameters in ISIAH rats in comparison with a group of adolescents with AH.

Material and Methods

We conducted the study on young (2.5-3 months) sexually mature male rats of the normotensive line (WAG) (n=20) (intact animals) and hypertensive line (ISIAH) (n=20), weighing 200-220 g. The animals were bred at the SPF-vivarium Center for collective use of the Federal research center "Institute of Cytology and Genetics," Siberian branch of RAS (Novosibirsk).[17] Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness). Blood was taken after rapid decapitation of the animals under anesthesia. The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations".

The data of 65 adolescents aged 13-17 years with AH (Group 1) were used for the clinical study (the age of adolescents is comparable to the biological age of the experimental animals used). The comparison group consisted of 65 normotensive adolescents of the same age and sex ("copy-pair" type) (Group 2). The common inclusion criterion for all groups was either voluntary informed consent of teenagers 15 years of age or older or of the parents/legal representatives of the adolescents.

The main inclusion criterion for Group 1 was confirmed AH based on the measurement of BP in repeated office measurements ≥95 percentile for age, height and sex or ≥140/90 mmHg in adolescents older than 16 years. Exclusion criteria for Group 1 were secondary hypertension and the presence of severe somatic diseases.

The main inclusion criteria for Group 2 were comparability by age, sex, place of residence and other basic criteria, as well as the presence of a normal BP level when measured 3 times, and the absence of acute disease or exacerbation of chronic diseases at the time of examination.

Exclusion criterion for all groups was intake of antioxidant drugs within the last 6 months.

Blood samples (5 ml) were collected from the ulnar vein in standard vacuum tubes with EDTA. The erythrocyte population was separated from the other blood components by centrifugation at 1500 *g* for 5 min, at 4°C. The erythrocyte pellet was washed 3 times with a 0.9% (wt/vol) NaCl solution. Aliquots of ethylenediaminetetraacetic acid plasma and washed erythrocytes were used immediately or kept frozen at -40°C, not exceeding one month. We estimated the LPO-AOD parameters by plasma concentrations of primary/secondary products of LPO (DB, CDs, KD-CT, and TBARs and antioxidant indexes (TAA, SOD activity, α-tocoferol, and retinol).[18] The concentration of CDs and KD-CT was detected at 232 nm in plasma heptane extracts. For conversion of absorption units to μmol/L, we used the coefficient of molar absorption ($K=2.2\cdot10^{5}M^{-1}C^{-1}$). TBARs levels were detected by fluorometry. Blood plasma total antioxidant activity (TAA) level was detected photometrically. α-tocopherol and retinol levels were detected in plasma by fluorometry. Fluorometry for SOD activity in hemolysate activity were determined. The measurements were conducted with a Shimadzu RF-1501 spectrophotometer (Japan) consisting of two blocks: a UV-1650PC spectrophotometer and a RF-1501 spectrofluorimeter.

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean±standard deviation (SD), median (Me) and interquartile range (IQR; 25th to 75th percentiles). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

At the first stage, we analyzed the data of the LPO-AOD system in ISIAH rats in comparison with WAG animals (Table 1).

In hypertensive rats, there was an increase in the average values of the final TBA-active LPO products (by 1.77 times; $P<0.001$) and a decrease in the activity of a number of antioxidant factors: lower values of TAA (by 1.72 times; $P<0.0001$), α-tocopherol (by 2.23 times; $P<0.0001$), and retinol (by 1.32 times; $P=0.026$) compared with WAG rats (Table 1).

Table 1.

The content of LPO products and components of AOD system in rats of the ISIAH line (M±SD, Me, IQR [P_{25}; P_{75}])

Parameters	WAG rats	ISIAH rats
Compounds with conjugated DB, units	1.74±0.35 1.81 1.54 – 1.87	1.88±0.19 1.38 1.76 – 2.07
CDs, μmol/L	0.94±0.31 0.86 0.73 – 0.89	1.16±0.13 1.14 1.12 – 1.22
KD-CT, units	0.34±0.04 0.34 0.31 – 0.37	0.36±0.08 0.36 0.31 – 0.38
TBARs, μmol/L	0.84±0.13 0.79 0.78 – 0.94	1.49±0.40* 1.66 1.07 – 1.83
TAA, units	16.45±2.71 17.14 14.36 –18.90	9.57±2.65* 10.34 8.29 – 10.52
SOD activity, units	2.06±0.24 1.98 1.94 – 2.14	2.16±0.37 2.02 1.84 – 2.51
α-tocopherol, μmol/L	10.91±2.55 11.20 9.85 – 12.30	4.89±1.55* 5.14 3.48 – 6.24
Retinol, μmol/L	0.54±0.15 0.51 0.48 – 0.65	0.41±0.08* 0.39 0.37 – 0.41

** - P<0.05 between two groups*

At the second stage, we analyzed the data of the LPO-AOD system in adolescents of Group 1 (Table 2) and discovered that in patients with AH, in relation to the data of Group 2, there was a statistically significant decrease in the value of primary LPO–CDs products by 1.34 times ($P=0.0028$) , as well as an increase in the content of intermediate LPO–KD-CT by 1.21 times ($P=0.0132$) and final TBA-active products by 1.43 times ($P<0.0001$). The state of the AOD system in Group 1 was characterized by a decrease in the α-tocopherol level by 1.38 times ($P<0.0001$), in the absence of changes in the values of TAA, SOD activity and retinol content (Table 2).

A high-brand line of rats with persistent ISIAH was determined to be the closest experimental model to humans for this pathological condition.[14,17] The ISIAH line is characterized by, among other things, an increase in BP at rest and a significant increase under mild emotional stress, the presence of specific morphological changes in organs and systems, an imbalance in the system of neuroendocrine regulation, and changes in behavioral reactions.[13] It is known that AH in humans is also associated with severe pathological conditions accompanied by numerous polysystem disorders, decreased immunity, early occurrence of atherogenic shifts, a significant imbalance of neurovegetative and endocrine influences, and significant changes in central and regional hemodynamics.[3,6]

Table 2.

The content of LPO products and components of AOD system in adolescents with AH (M±SD, Me, IQR [P_{25}; P_{75}])

Parameters	Comparison group	Group of adolescents with AH
Compounds with conjugated DB, units	1.5±0.48 1.48 1.08 – 1.84	1.47±0.63 1.39 0.99 – 1.84
CDs, μmol/L	1.42±0.7 1.44 0.75 – 2.06	1.06±0.67* 0.98 0.52 – 1.56
KD-CT, units	0.33±0.17 0.28 0.2 – 0.4	0.4±0.17* 0.36 0.28 – 0.48
TBARs, μmol/L	0.73±0.29 0.73 0.49 – 0.87	1.04 ±0.5* 0.91 0.65 – 1.38
TAA, units	13.33±4.14 13.12 10.79 – 15.75	14.96±6.33 14.26 10.34 – 19.22
SOD activity, units	1.56±0.18 1.57 1.44 –1.72	1.56±0.17 1.58 1.43 – 1.67
α-tocopherol, μmol/L	7.66±3.33 7.32 5.25 – 9.00	5.56±2.3* 5.06 4.06 – 6.27
Retinol, μmol/L	0.8±0.33 0.73 0.56 – 0.99	0.9±0.41 0.86 0.62 – 1.12

** - P<0.05 between two groups*

When analyzing the experimental data, we found that rats of the ISIAH line have a high activity of LPO reactions (an increase in the final TBA-active products). Our data are consistent with the results of a number of studies indicating the altered reactivity of various systems in animals of this line.[13] Thus, characteristic violations in the biochemical status of the animals, indicating an increase in the level of cholesterol-containing fractions of lipids, glucose, lactic acid, etc., were revealed earlier.[19] It can be assumed that these disorders can directly affect the state of nonspecific bodily reactivity systems, provoking the development of OS in hypertensive animals. Thus, it is possible to point to the presence of a significant shift toward prooxidant activity in rats of the ISIAH line in comparison with WAG rats.

In the analysis of the primary link of the LPO process in AH adolescents that includes the formation of compounds with unsaturated substrates with conjugated double bond and primary products – CDs. Previously, the presence of LPO activation had not been revealed, since the value of the CDs was lower than in Group 2. In this case, it is legitimate to note an increase in the rate of transition of the primary LPO products to subsequent metabolites, especially since changes in the secondary link showed a statistically significant increase in the content of intermediate LPO products - KD-CT. The activation of LPO processes can be judged by a significant

increase in the concentration of final TBA-active LPO products. The increase of secondary and final LPO metabolites in AH adolescents may be a sufficient criterion for the conclusion about the activation of free radical reactions, especially since a variety of LPO products, including TBA-active LPO products, have a multilateral damaging effect on most biopolymers and cell structures.[20,21] According to some data in the literature, a possible explanation for the growth of reactive LPO products in hypertension may be an imbalance in the neuroendocrine regulation system, resulting in systemic metabolic disorders with atherogenic changes in blood composition.[22-24] In addition, the development of this disease provoked changes in the fatty acid composition of blood plasma lipids: an increase in the total content of saturated and monounsaturated fatty acids with a reduction in the concentration of polyunsaturated components.[8] The consequence of such destabilization is the development of OS in patients with AH. With respect to the studied problem, it should also be noted that the generation of toxic LPO products is closely related to the synthesis of nitric oxide, the concentration of which positively correlates with the activity of an angiotensin converting enzyme, which is one of the most important factors in the regulation of BP.[6,11] Having a free radical structure, as well as functioning as a signaling molecule of the cardiovascular system, nitric oxide has a significant regulatory effect on the system's activity, supporting vasodilation at the required level and regulating regional hemodynamics. Thus, the activation of LPO reactions can lead to serious consequences, including at the level of the vascular bed.

The limiting factor of the LPO processes in the body is the high activity of antioxidant factors that make up the overall antioxidant status.[8,11] It was found that the increased values of toxic metabolites in hypertensive animals took place against the background of a significant decrease in antioxidant protection factors—TAA and the content of fat-soluble vitamins. It is known that α-tocopherol is a strong antioxidant of exogenous origin.[25] The mechanism of its action is due to the ability of the mobile hydroxyl of the vitamin molecule chromane nucleus to interact directly with free radicals: active radicals of oxygen, unsaturated fatty acids and their peroxides.[26] It is likely that the accumulation of toxic metabolites in ISIAH rats may be due to insufficient activity of antioxidant factors.

In AH, adolescents had an imbalance of the LPO-antioxidant system, with changes in the level of antioxidant components. Thus, in the AH group, there was a significant decrease in the α-tocopherol value in the absence of changes in the values of TAA, SOD activity, and the level of non-enzymatic antioxidants. It is known that due to the lipophilic properties, the α-tocopherol molecule has the ability to be embedded in the lipid bilayer of cell membranes and thus have a membrane-protective and membrane-stabilizing effect. In addition, this fat-soluble vitamin supports the functional stability of the external plasma membrane of cells and participates in the regulation of tissue respiration in mitochondria and the work of cell enzyme systems that interfere with the LPO activity.[27] The decrease in the concentration of α-tocopherol in the blood of patients is the evidence of stress in the AOD system, which is confirmed by a significant accumulation of intermediate and final LPO products.

Conclusion

Thus, changes in nonspecific mechanisms of bodily reactivity in animals and humans under conditions of AH indicate the development of a predominantly unidirectional disadaptive reaction, reflecting the prevalence of pathogenetic reactions (accumulation of prooxidant factors) over sanogenetic (antioxidant defense activation) ones. Species differences in this case consist in the difference in the intensity of reactions and the number of parameters involved in the development of the pathological process. We can talk about the formation of stable pathological reactions in animals (accumulation of final metabolites), while in humans there is a dynamics of the pathological process (growth of intermediate and final metabolites). Reactivity of the AOD system in animals was characterized by a more pronounced decrease in protective reserves, whereas in humans there were changes in a single antioxidant parameter. Thus, animals of the ISIAH line can be a genetic model for the study of new mechanisms of the body's adaptive reserves under the influence of AH; however, a number of differences have been identified that require consideration in the studies of AH in the experiment in order to develop methods of treatment and optimization of sanogenetic mechanisms aimed at leveling pathological effects in patients with AH.

Competing Interests

The authors declare that they have no competing interests.

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