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ВЛИЯНИЕ АРГИНИНА НА АКТИВНОСТЬ И КОМПАРТМЕНТАЛИЗАЦИЮ ЛИЗОСОМАЛЬНЫХ ЦИСТЕИНОВЫХ ПРОТЕИНАЗ ПАРЕНХИМАТОЗНЫХ ОРГАНОВ ПРИ ОКСИДАТИВНОМ СТРЕССЕ НА ФОНЕ ЭКСПЕРИМЕНТАЛЬНОЙ ГИПЕРГОМОЦИСТЕИНЕМИИ

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Цель. Оценка влияния аргинина на активность и внутриклеточное распределение катепсинов B, L, H в ткани печени, почки и легкого при экспериментальной гипергомоцистеинемии и развивающегося на ее фоне окислительного повреждения белков. ***Материалы и методы.*** Гипергомоцистеинемию у крыс-самцов линии *Wistar* формировали пероральным введением суспензии метионина в дозе 1,5 г/кг ежедневно 2 раза в сутки в течение 21 суток, для изучения действия аргинина субстанцию в дозе 500 мг/кг применяли перорально с 12 по 21 сутки введения метионина. В гомогенатах тканей измерения проводились в цитоплазматической и лизосомальной фракциях. Оценка состояния окислительной модификации белков проводилась анализом спектра поглощения карбонильных производных, активность катепсинов B, L, H – спектрофлуориметрическим методом, активность кислой фосфатазы – унифицированным методом «по конечной точке». ***Результаты.*** В цитоплазматической (неседиментируемой) фракции печени и почки на фоне нарастания продуктов окислительной модификации белков при экспериментальной гипергомоцистеинемии обнаружено снижение активности катепсина L (в обеих тканях), катепсина B (в почке), катепсина H (в печени). Введение аргинина при экспериментальной гипергомоцистеинемии полностью устраняло проявления окислительного повреждения белков, частично корректируя активность ферментов: наблюдалось нарастание активности в неседиментируемой (цитоплазматической) фракции за счет внутриклеточного перераспределения ферментов. Обнаружены обратные корреляционные связи между содержанием продуктов окислительного карбонилирования белков и активностью катепсинов в неседиментруемой фракции, а также долей их неседиментируемой активности. ***Выводы.*** 1. Аргинин в дозе 500 мг/кг при 10-дневном введении полностью корректирует развивающееся на фоне экспериментальной гипергомоцистеинемии повышение продуктов окислительного карбонилирования белков. 2. Под действием аргинина происходит нарастание сниженной при изолированной гипергомоцистеинемии активности катепсинов B, L, H в цитоплазматической фракции печени и почки за счет внутриклеточного перераспределения ферментов. 3. Изменения компартментализации лизосомальных цистеиновых протеиназ под действием аргинина происходят через вызываемое им неселективное повышение проницаемости лизосомальной мембраны. 4. Обнаружены обратные корреляционные связи содержания продуктов окислительной модификации белков с активностью катепсинов в цитоплазматической (неседиментируемой) фракции и долей их неседиментируемой активности, позволяющие предполагать наличие

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вклада изменения компартментализации лизосомальных цистеиновых протеиназ в развивающуюся под действием аргинина компенсацию окислительного стресса на фоне экспериментальной гипергомоцистеинемии.

Ключевые слова: *гипергомоцистеинемия, аргинин, катепсины B, L, H, окислительная модификация белков.*

THE EFFECT OF ARGININE ON THE ACTIVITY AND COMPARTMENTALIZATION OF LYSOSOMAL CYSTEINE PROTEINASES OF PARENCHYMATOUS ORGANS IN OXIDATIVE STRESS ON THE BACKGROUND OF EXPERIMENTAL HYPERHOMOCYSTEINEMIA

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Aim. Evaluation of the effect of arginine on the activity and intracellular distribution of cathepsins B, L, H in liver, kidney and lung tissue in experimental hyperhomocysteinemia and developing on its background oxidative damage of proteins. ***Materials and Methods.*** Hyperhomocysteinemia in male rats of the *Wistar* line was formed by daily oral administration of a suspension of methionine at a dose of 1.5 g/kg 2 times a day for 21 days, to study the action of arginine the substance was used orally from 12 to 21 days of methionine administration at a dose of 500 mg/kg. In tissue homogenates measurements were carried out in cytoplasmic and lysosomal fractions. The state of oxidative modification of proteins was evaluated by analysis of the absorption spectrum of carbonyl derivatives, the activity of cathepsins B, L, H was determined by spectrofluorometric method, the activity of acid phosphatase – by the unified method «at the end point». ***Results.*** In the cytoplasmic (nonsedimentary) fraction of the liver and kidney reduced activity of cathepsin L (in both tissues), cathepsin B (in the kidney), cathepsin H (in the liver) was found on the background of the increase in the products of proteins oxidative modification in experimental hyperhomocysteinemia. The introduction of arginine in experimental hyperhomocysteinemia completely eliminated the manifestations of oxidative damage of proteins, partially correcting the activity of enzymes: there was an increase in the activity in nonsedimentary (cytoplasmic) fraction due to intracellular redistribution of enzymes. There is found inverse correlation between the content of oxidative carbonylation products of proteins and the activity of cathepsins in the non- sedimentary fraction, as well as the proportion of their nonsedimentary activity. ***Conclusions.*** 1. Arginine at a dose of 500 mg/kg at a 10-day administration completely corrects the increase in the products of oxidative protein carbonylation that develops in experimental hyperhomocysteinemia. 2. Under the influence of arginine there is an increase in the reduced due to isolated hyperhomocysteinemia activity of cathepsins B, L, H in the cytoplasmic fraction of the liver and kidney due to intracellular redistribution of enzymes. 3. Arginine administration causes non-selective increase of the lysosomal membrane permeability, and as a result, changes in the compartmentalization of lysosomal cysteine proteases. 4. The inverse correlation of the level of protein oxidative modification products with the activity of cathepsins in cytoplasmic (nonsedimentary) fractions, and the proportions of their nonsedimentary activity, suggesting the presence of contribution of changes in the compartmentalization of lysosomal cysteine proteinases in developing under the action of arginine compensation of oxidative stress in experimental hyperhomocysteinemia.

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Keywords: *hyperhomocysteinemia, arginine, cathepsins B, L, H, oxidative modification of proteins.*

Homocysteine is an intermediary product of methionine metabolism that possesses an evident cytotoxic effect realized through several ways including provocation of production of free radicals leading to oxidative stress [1]. At present the most significant manifestation of oxidative stress is considered to be oxidative modification of proteins, since this process not only causes formation of stable markers convenient for measurement and further interpretation, but considerably changes different functions of these biomolecules [2] leading to disorders in the functions of cells and tissues with the underlying free-radical pathology. The highest significance is attributed to irreversible oxidation of proteins with production of carbonyl derivatives [3]. Such proteins lose their native functions and form aggregates subject to degradation. The agents of such degradation may be different kinds of cellular and extracellular proteinases including lysosomal ones. Of highest interest are lysosomal cysteine proteinases due to proven ability not only to breakdown proteins captured in endocytosis, but also to move to the cytoplasm across excessively permeable lysosomal membrane [4]. It should be noted that despite a considerable amount of works that connect increase in the level of homocysteine in blood with development of different pathological conditions, the specification of mechanisms of changes in tissue still remains important. Since in the previous studies we found changes in the activity of lysosomal cysteine proteinases and in permeability of lysosomal membranes in the myocardium in hyperhomocysteiemia and showed the correcting effect of arginine on the above mentioned processes [5], the aim of this research was evaluation of the influence of arginine on the activity and intracellular distribution of cathepsins B, L, H in the liver, kidney and lung tissue in experimental hyperhomocysteinemia with the resultant oxidative damage to proteins.

Materials and Methods

The study was carried out on 32 conventional mature male rats of *Wistar* line. The animals were managed and withdrawn from the experiment according to the protocols stated by Council of International Organizations of Medical Sciences (CIOMS) in «International recommendations on medico-biological research with use of animals» (1985) and to Rules of laboratory practice – Addendum to The Order of Ministry of Health and Social Development of Russian Federation №708н of August 23, 2010.

Evident hyperhomocysteinemia [6] was modeled by intragastric introduction of methionine as a substrate for synthesis of homocysteine, in the form of suspension prepared on the basis of 1% starch solution with addition of 10% Tween 80 at a dose of 1.5 g/kg twice a day within 21 days (n=80). The suspension was introduced by an experimenter with participation of an assistant using a glass graduated syringe with a probe. The volume of the introduced suspension depended on the mass of the animal and did not exceed 1.5 ml. The animals were withdrawn from the experiment on the 22nd day.

The animals of the control group (n=8), control 1, were in the same way (intragastrically) introduced the suspension that did not contain methionine (composition by mass: 10% Tween 80, 1% starch, 89% of water) within 21 days. The animals were withdrawn from the experiment on the 22nd day.

To study the effect of arginine with the underlying expressed hyperhomocysteinemia a sample of animals (n=8) was introduced arginine *per os* 1 time a day at a dose 500 mg/kg on the basis of 0.9% NaCl solution within 10 days [7] in parallel with the introduction of methionine according to the above described scheme (from the 12th to 21st day of introduction of methionine).

The animals of the control group (n=8) were introduced arginine *per os* 1 time a day

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at a dose of 500 mg/kg based on 0.9% NaCl solution within 10 days in parallel with the introduction of suspension not containing methionine, according to the above described scheme – control 2. The animals were withdrawn from the experiment on the 22nd day.

The animals were withdrawn from the experiments by euthanasia under ether narcosis by exsanguination with preserved respiration and heart beating, after which the liver, kidney and lung were immediately extracted. The organs were separately placed into a cooled 0.25 M solution of sucrose, were cleared of the remaining adipose and connective tissue, were washed in 0.25 M solution of sucrose, and precise weighted quantities were prepared using electronic scales (AJH-220, Japan). Homogenization of tissues was carried out in a cold 0.25 M solution of sucrose in proportion 1:10 in a «Potter S» homogenizer (Sartorius, Germany) in a glass utensil with a Teflon pounder with the gap of 0.16-0.24 mm. The rotation speed was 1000 rev/min for the kidney and lung and 900 rev/min for the liver. The time of homogenization was 50 and 35 sec, respectively.

Subcellular fractionation of the obtained homogenates was carried out by sequential centrifugation: 15 min at 800 g for elimination of incompletely destructed cells and nuclei, 10 min at 14000 g for elimination of mitochondria and 30 min at 20000 for sedimentation of lysosomes. The supernatant obtained in the final centrifugation was a cytoplasmic (nonsedimentary) fraction of homogenate, sediment (sedimentary fraction) – a coarse fraction of lysosomes. For further study the sediment was resuspended in 0.25 M sucrose with addition of Triton X-100 in the final concentration 0.1%.

Concentration of homocysteine was determined in blood serum using a commercial kit «AxisShield» (США) by the method of immune enzyme assay, concentration of protein in nonsedimentary and sedimentary fractions of homogenates – by Lowry method using a commercial kit of SPC «Eco-service» (Russia, St.-Petersburg).

To evaluate the evidence of oxidative modification of proteins, the level of carbonyl derivatives was measured in nonsedimentary fraction of homogenates by method of R.L. Levine in modification of E.E. Dubinina in spontaneous and metal-induced variants [8] with registration of the products of reaction (dinitrophenyl hydrazones) on spectrophotometer (СФ-2000, Russia, St.-Petersburg) in the ultraviolet and visible spectra. The obtained results were analyzed by the method of comprehensive assessment of the content of products of oxidative modification of proteins [9]. The results were expressed in units of optic density per gram of protein. Reserve-adaptation potential (RAP) [10] was evaluated using the values of the total areas under the curve of the absorption spectrum of carbonyl derivatives, with presentation of the result in percent.

The activities of lysosomal cysteine proteinases – cathepsins B, L and H were studied separately in the sedimentary and nonsedimentary fractions of homogenates by spectrofluorometric method [11] with registration of the product of reaction (7-amido-4-methylcoumarin) on spectrofluorometer System 3 Scanning Spectrofluorometr (Optical technology devices, Inc. Elmstord, New York, 10523). Specific activity of enzymes was expressed in ncat/g of protein and was designated NSA (for nonsedimentary fraction) and SA (for sedimentary). The total activity of homogenate (TA) was calculated as the sum of NSA and SA for each enzyme.

For evaluation of the compartmentalization of cathepsins the value of the share of extralysosomal activity was used (NSA%) which was calculated as percentage of nonsedimentary activity of the respective enzyme in its total activity.

The autocatalytic action of cathepsins was evaluated by coefficient of the ratio of the value of activity of each enzyme after 15-min precalatytic incubation to the simultaneously determined activity without preincubation [12] (K_{aca} – coefficient of autocatalytic action).

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As an additional marker of stability of the lysosomal membrane in sedimentary and nonsedimentary fractions of homogenates the activity of acid phosphatase (total and tartrate-stable, APtst) was determined using a commercial kit «Vital Diagnostics SPb (Russia, St.-Petersburg), activity of tartrate-sensitive fraction (APtsen) was determined as a difference between the total and tartrate-stable activity.

Statistical processing of the results was carried out using Statistica 10.0 program. Normality of distribution of the data was checked using Shapiro-Wilk test (W-test). Since there was no agreement between the majority of data and normal distribution, median (Me), upper and lower quartiles (Q1 and Q3, respectively) were used as characteristics, with presentation of the results in Me [Q1;Q3] format. Statistical significance of differences between independent samples was evaluated using Mann-Whitney U-test (U-test). Rank order correlation was evaluated using Spearman coefficient.

Results and Discussion

Measurement of concentration of homocysteine in blood serum demonstrated a significant increase in the parameter in animals who received methionine as compared to control group 1 (293.10 [273.10; 318.20] μm/L and 5.90 [5.50; 6.70] μmol/L, respectively, p=0.001). Here, in animals who were given arginine with the introduction of methionine this parameter was 92.80 [58.75; 112.07] that showed a statistically significant reduction compared to parameters of the group with isolated introduction of methionine (p=0.001), but was considerably higher than in control group 2 (5.87 [5.65; 6.77], p=0.002).

Comprehensive evaluation of oxidative modification of proteins in the studied tissues (Table 1) showed a statistically significant increase in the total concentration of oxidatively carbonylated protein in liver and kidney (S_{tot}) in experimental hyperhomocysteinemia due to statistically significant increase in the concentration of aldehyde forms of dinitrophenylhydrazones (ADNPH) considered to be the primary markers of oxidative damage to proteins [13]. Thus, this confirms the thesis about the role of homocysteine as a provoker of oxidative stress in the mentioned tissues, besides, our data agree with the similar results for the myocardium [14]. However, it should be noted that unlike the myocardium, the hepatic and renal tissues showed neither statistically significant changes in the secondary markers of oxidative damage to proteins (ketodinitrophenylhydrazones, KDNPH), nor changes in RAP parameter. So despite the evident signs of development of oxidative stress in tissues of liver and kidney in hyperhomocysteinemia, it was less expressed in them in comparison with the cardiac muscle. This may be due to both a better developed system of antioxidant protection, and better utilization of proteins with the primarily oxidative modification.

Introduction of arginine at a dose 500 mg/kg within 10 days with the underlying introduction of methionine led to a complete correction of the identified changes and brought the parameters of oxidative carbonylation of proteins close to the parameters of the respective control group, and formed statistically significant reductions in the levels of S_{tot} and S_{ADNPH} relative to the group that received methionine.

Evaluation of changes in the activity and intracellular distribution of lysosomal cysteine proteinases in the oxidative stress induced by experimental hyperhomocysteinemia, and its correction by arginine, revealed a number of tissue- and enzyme-specific tendencies.

Thus, cathepsin B (Table 2) showed the most evident differences in tissues of kidney: the activity of the enzyme in hyperhomocysteinemia revealed significant reduction in both lysosomal (SA) and in the cytoplasmic (NSA) fractions with a statistically significant reduction in the total activity (TA).

Here, introduction of arginine caused only increase in the activity of the enzyme in the cytoplasmic fraction, having formed statistically significant increase in comparison with parameters both of the group that received methionine and of the respective control group. The absence of statistically signi-

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Table 1

Results of Comprehensive Assessment of Conditions of Oxidative Modifications of Proteins in Experimental and Control Groups (Me [Q_1; Q_3])

Parameter	Group/Organ		
	Control 1		
	Liver	Kidney	Lung
S_{tot}	2,87 [2,76;2,87]	2,22 [2,12;2,64]	2,10 [1,69;2,82]
$S_{ADNPH, sum}$	2,34 [2,07;2,53]	1,83 [1,72;2,19]	1,61 [1,28;2,27]
$S_{RDHPH, sum}$	0,60 [0,46;0,76]	0,41 [0,32;0,46]	0,47 [0,41;0,54]
RAP%	57,10 [49,50; 57,10]	50,00 [42,20; 54,50]	37,80 [35,80; 46,00]
	Methionine		
	Liver	Kidney	Lung
S_{tot}	5,30 [4,25;5,50]*, p_1=0,003	3,58 [2,94;3,72]*, p_1=0,005	2,19 [1,90;2,81]
$S_{ADNPH, sum}$	4,75 [3,85;5,16]*, p_1=0,002	2,93 [2,44;3,02]*, p_1=0,003	1,67 [1,51;2,11]
$S_{KDNPH, sum}$	0,42 [0,35;0,52]	0,55 [0,40;0,71]	0,41 [0,39;0,53]
RAP%	58,10 [29,40;59,10]	48,8 [44,80; 67,90]	54,30 [44,80; 65,20]
	Control 2		
	Liver	Kidney	Lung
S_{tot}	1,03 [0,99;1,07]	2,41 [1,06;3,90]	2,16 [1,98;2,47]
$S_{ADNPH, sum}$	0,71 [0,69;0,73]	2,10 [0,66;3,38]	1,67 [1,57;1,98]
$S_{KDNPH, sum}$	0,33 [0,28;0,35]	0,45 [0,41;0,54]	0,44 [0,40;0,55]
RAP%	24,5 [22,50;26,90]	54,50 [38,20;70,00]	49,60 [36,90;56,40]
	Methionine+Arginine		
	Liver	Kidney	Lung
S_{tot}	1,46 [1,03;1,89]#, p_2=0,008	1,85 [1,45;2,42]#, p_2=0,047	1,92 [1,49;2,34]
$S_{ADNPH, sum}$	1,21 [0,81;1,57]#, p_2=0,008	1,60 [1,31;1,93]#, p_2=0,03	1,26 [1,11;1,83]
$S_{KDNPH, sum}$	0,25 [0,23;0,32]#, p_2=0,05	0,35 [0,23;0,50]	0,53 [0,38;0,63]
RAP%	67,10 [50,27;76,00]	76,30 [59,30;80,10]	54,30 [23,50;80,50]

Note: *, p_1 – statistically significant differences from the respective control group, #, p_2 – statistically significant differences from the group receiving methionine

ficant differences in the total activity of the enzyme in combination with a considerable increase in the share of nonsedimentary activity (NSA%) shows that the cause is alteration of compartmentalization with intensification of the release of the enzyme from the lysosomal to cytoplasmic fraction for utilization of proteins with oxidative damage. The liver tissue also showed a tendency to reduction in the activity of cathepsin B in hyperhomocysteinemia, however, statistically significant changes were obtained only for nonsedimentary activity. It is interesting that introduction of arginine in this case not only does not correct but even worsens the identified changes increasing a statistically significant difference from the parameters of control group without statistically significant differences from the group with isolated hyperhomocysteinemia.

Changes in the activity and compartmentalization of cathepsin L (Table 3) in experimental hyperhomocysteinemia were similar to those of cathepsin B but were much more expressed. Thus, statistically significant reduction in NSA, SA and TA of cathepsin L was found both in the tissues of kidney and liver, here, in both tissues the reduction of the

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Table 2

Changes in Activity and Compartmentalization of Lysosomal Cysteine Proteinases in Experimental and Control Groups: Cathepsin B (Me [Q_1; Q_3])

Parameter	Group/Organ		
	Control 1		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	0,43 [0,39;0,54]	0,93[0,85;1,12]	0,14 [0,12;0,17]
SA, ncat/g of protein	0,41 [0,38;0,52]	0,88[0,81;1,08]	0,14 [0,12;0,16]
NSA, ncat/g of protein	0,020 [0,015;0,024]	0,045 [0,042;0,047]	0,003 [0,003;0,005]
NSA, %	4,30 [2,9;5,6]	4,9 [4,0;5,6]	2,6 [1,9;4,6]
Kidney	**Methionine+Arginine**		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	0,37 [0,29;0,43]	0,64 [0,50;0,81]*, p_1=0,01	0,16 [0,04;0,24]
SA, ncat/g of protein	0,36 [0,29;0,42]	0,62 [0,48;0,79]*, p_1=0,02	0,16 [0,04;0,24]
NSA, ncat/g of protein	0,007 [0,005;0,011]*, p_1=0,01	0,020 [0,016;0,022]*, p_1=0,002	0,003 [0,001;0,003]
NSA, %	2,4 [1,2;3,2]	3,2 [2,6;4,6]	1,7 [1,1;3,1]
	Control 2		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	0,34 [0,30;0,40]	1,36 [0,68;2,07]	0,94 [0,81;1,06]
SA, ncat/g of protein	0,33 [0,29;0,38]	1,31 [0,62;2,02]	0,93 [0,80;1,05]
NSA, ncat/g of protein	0,011 [0,010;0,012]	0,058 [0,050;0,067]	0,011 [0,010;0,012]
NSA, %	3,0 [2,7;3,4]	4,0 [2,8;9,5]	0,9 [0,7;1,4]
	Methionine+Arginine		
	Liver	**Почка**	**Lung**
TA, ncat/g of protein	0,23 [0,20;0,24]*, p_1=0,005	0,59 [0,50;0,67]	0,98 [0,92;1,13]#, p_2=0,002
SA, ncat/g of protein	0,22 [0,20;0,24]*, p_1=0,005	0,51 [0,43;0,59]	0,97 [0,92;1,12]#, p_2=0,002
NSA, ncat/g of protein	0,005 [0,005;0,006]*, p_1=0,005	0,072 [0,071;0,088]*#, p_1=0,01, p_2=0,002	0,005 [0,002;0,008]
NSA, %	2,3 [2,0;2,6]*, p_1=0,05	13,8 [11,3;14,3]#, p_2=0,002	0,4 [0,1;1,1]#, p_2=0,05

Note: *, p_1 – statistically significant differences from the respective control group, #, p_2 – statistically significant differences from the group receiving methionine

activity was more expressed in the cytoplasmic than lysosomal fraction which was confirmed by statistically significant changes in the share of nonsedimentary activity.

Intake of arginine again did not correct changes in the total and lysosomal activity of cathepsin L in the hepatic tissue, like of cathepsin B, however, it caused redistribution of the enzyme into the cytoplasmic fraction leading to statistically significant increase in the nonsedimentary activity of cathepsin L and in its share relative to the group that received only methionine. But introduction of arginine with methionine led to a partial correction of changes in the total and lysosomal activity of cathepsin L in kidney tissue: the value appeared statistically higher than in the group that received methionine alone, but statistically lower than in the corresponding control group. Here, changes in the activity of enzyme in the nonsedimentary fraction were completely corrected, and again this may be attributed to redistribution of the enzyme: the share of nonsedimentary activity showed a

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Таблица 3

Changes in Activity and Compartmentalization of Lysosomal Cysteine Proteinases in Experimental and Control Groups: Cathepsin L (Me [Q_1; Q_3])

Parameter	Group/Organ		
	Control 1		
	Liver	Kidney	Lung
TA, ncat/g of protein	1,70 [1,19;2,10]	2,03 [1,90;2,27]	1,65 [1,45;1,84]
SA, ncat/g of protein	1,64 [1,14;2,03]	1,97 [1,83;2,18]	1,64 [1,40;1,83]
NSA, ncat/g of protein	0,063 [0,061;0,066]	0,071 [0,068;0,079]	0,019 [0,014;0,026]
NSA, %	3,9 [3,1;5,1]	3,8 [3,1;4,0]	1,3 [0,8;1,5]
	Methionine		
	Liver	Kidney	Lung
TA, ncat/g of protein	1,12 [0,93;1,22]*, p_1=0,04	0,66 [0,55;0,74]*, p_1=0,001	2,05 [1,73;2,31]
SA, ncat/g of protein	1,10 [0,91;1,21]	0,66 [0,54;0,73]*, p_1=0,001	2,01 [1,72;2,31]
NSA, ncat/g of protein	0,019 [0,010;0,025]*, p_1=0,001	0,009 [0,007;0,013]*, p_1=0,001	0,021 [0,006;0,035]
NSA, %	2,0 [0,8;2,4]*, p_1=0,001	1,2 [1,1;1,6]*, p_1=0,001	0,9 [0,4;1,8]
	Control 2		
	Liver	Kidney	Lung
TA, ncat/g of protein	1,16 [1,03;1,28]	2,92 [2,37;4,12]	1,70 [1,31;2,18]
SA, ncat/g of protein	1,12 [0,99;1,25]	2,83 [2,30;4,06]	1,68 [1,30;2,15]
NSA, ncat/g of protein	0,037 [0,028;0,042]	0,075 [0,071;0,084]	0,024 [0,020;0,026]
NSA, %	3,1 [2,5;3,4]	2,8 [1,9;3,5]	1,2 [0,9;1,4]
	Methionine+Arginine		
	Liver	Kidney	Lung
TA, ncat/g of protein	1,23 [1,00;1,27]	1,89 [1,82;1,94]*#, p_1=0,02, p_2=0,002	1,52 [1,33;1,99]
SA, ncat/g of protein	1,18 [0,95;1,23]	1,81 [1,75;1,86]*#, p_1=0,01, p_2=0,002	1,50 [1,30;1,98]
NSA, ncat/g of protein	0,048 [0,040;0,054]#, p_2=0,008	0,080 [0,074;0,084]#, p_2=0,002	0,026 [0,016;0,034]
NSA, %	4,0 [3,1;5,4]#, p_2=0,002	4,0 [3,9;4,3]*#, p_1=0,005, p_2=0,01	1,6 [1,0;2,5]

Note: *p_1 – statistically significant differences from the respective control group, #, p_2 – statistically significant differences from the group receiving methionine

statistically significant increase not only relative to the group with experimental hyperhomocysteinemia, but also relative to the corresponding control group.

Changes in the activity and distribution of cathepsin H (Table 4) in the liver tissue practically completely repeated changes found for cathepsin L with the only difference being incomplete correction of reduction in the nonsedimentary activity by arginine. Here, in experimental hyperhomocysteinemia we found increase in the total activity of the enzyme in the kidney tissue, however, these changes occurred due to lysosomal fraction, the share of nonsedimentary activity appeared to be statistically below the control values. Nevertheless, application of arginine caused additional increase in the total activity of the enzyme which statistically exceeded the values for the group with isolated introduction of

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Table 4

Changes in Activity and Compartmentalization of Lysosomal Cysteine Proteinases in Experimental and Control Groups: Cathepsin H (Me [Q_1; Q_3])

Parameter	Group/Organ		
	Control 1		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	0,75 [0,56;0,83]	1,63 [1,26;2,29]	1,24 [1,11;1,46]
SA, ncat/g of protein	0,70 [0,52;0,78]	1,57 [1,21;2,24]	1,21 [1,08;1,42]
NSA, ncat/g of protein	0,048 [0,037;0,059]	0,044 [0,043;0,070]	0,039 [0,024;0,044]
NSA, %	7,1 [6,4;7,3]	3,5 [2,3;4,3]	2,9 [2,6;3,1]
	Methionine		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	0,50 [0,44;0,54]*, p_1=0,01	2,64 [2,52;2,78]*, p_1=0,02	1,45 [1,19;1,78]
SA, ncat/g of protein	0,47 [0,41;0,51]*, p_1=0,02	2,60 [2,48;2,75]*, p_1=0,01	1,43 [1,16;1,75]
NSA, ncat/g of protein	0,028 [0,025;0,030]*, p_1=0,004	0,038 [0,032;0,051]	0,024 [0,022;0,031]
NSA, %	5,6 [5,0;6,3]*, p_1=0,01	1,5 [1,2;2,0]*, p_1=0,02	2,0 [1,1;2,5]
	Control 2		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	1,06 [0,91;1,26]	3,49 [3,27;4,14]	3,34 [2,88;3,56]
SA, ncat/g of protein	0,98 [0,84;1,18]	3,40 [3,20;4,07]	3,24 [2,79;3,47]
	0,081 [0,073;0,087]	0,080 [0,071;0,084]	0,094 [0,083;0,097]
NSA, %	7,6 [6,9;7,8]	2,2 [1,8;2,5]	2,8 [2,6;3,0]
	Methionine+Arginine		
	Liver	**Kidney**	**Lung**
TA, ncat/g of protein	0,93 [0,89;1,08]#, p_2=0,002	3,01 [2,97;3,05]#, p_2=0,05	2,32 [2,07;2,48]*#, p_1=0,008, p_2=0,008
SA, ncat/g of protein	0,85 [0,83;1,00]#, p_2=0,002	2,92 [2,88;2,96]	2,26 [2,01;2,41]*#, p_1=0,008, p_2=0,01
NSA, ncat/g of protein	0,070 [0,068;0,072]*#, p_1=0,05, p_2=0,002	0,088[0,084;0,090]*#, p_1=0,05, p_2=0,002	0,069[0,061;0,077]*#, p_1=0,02, p_2=0,002
NSA, %	7,3 [6,4;8,2]#, p_2=0,02	2,9 [2,8;3,0]#, p_2=0,002	3,0 [2,8;3,3]#, p_2=0,02

Note: *p_1 – statistically significant differences from the respective control group, #, p_2 – statistically significant differences from the group receiving methionine

methionine and approached the values of the corresponding control. Especially evident were changes in the nonsedimentary activity, that statistically exceeded not only values for the group with isolated introduction of methionine, but also the parameters of the control group. The contribution of the intracellular redistribution of the enzyme into the identified changes was confirmed by statistically significant increase in the share of the cytoplasmic fraction relative to the group with experimental hyperhomocysteinemia.

It is interesting that the lung tissue demonstrated resistance to development of oxidative stress in experimental hyperhomocysteinemia, and the absence of changes in the parameters of the oxidative damage to proteins in this tissue were accompanied by the absence of changes in the activity and intracellular distribution of lysosomal cysteine proteinases.

In evaluation of the parameters of oxidative carbonylation of proteins and activity/distribution of cathepsins for the studied

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models the most vivid tendencies were obtained for kidney tissue, where the changes in whole also appeared the most expressed (Table 5). For all studied enzymes there were obtained statistically significant inverse correlations between the total content of carbonylated proteins, primary markers of oxidative damage to proteins, and the parameters of nonsedimentary activity of all studied cathepsins and shares of cytoplasmic fraction for both all cathepsins and acid phosphatase. The extent of correlation relationship in all cases was from medium (0.55) to high (0.87).

In the liver tissue statistically significant correlation relationships with parameters of oxidative damage to proteins were obtained only for the activity of cathepsin H: nonsedimentary activity showed inverse correlation of the medium extent (Fig. 1), sedimentary and total activity – inverse correlation of high extent (Fig. 2 and 3, respectively). Here, for the share of cytoplasmic fraction inverse correlations were obtained for cathepsin B (-0,55, p=0,01 with S_{tot}; -0,56, p=0,01 with $S_{ADNPH,\ sum}$), cathepsin L (-0,49, p=0,02 with S_{tot}; -0,47, p=0,03 with $S_{ADNPH,\ sum}$), and for tartrate-sensitive acid phosphatase, APtsen (-0,46, p=0,03 with S_{tot}; -0,45, p=0,04 with $S_{ADNPH,\ sum}$).

The obtained results confirm existence of the relationship between the change in the activity of lysosomal enzymes, their redistribution into the cytoplasmic fraction and the level of oxidative damage to proteins. The inverse character of correlations may be considered a particular confirmation of the general thesis about inhibitory influence of oxidative modification of proteins on their functions, including enzymatic ones. At the same time existence of such relationships permits to suggest that correction of parameters of oxidative modification of proteins under influence of arginine may be due to simultaneously occurring movement of lysosomal cysteine proteinases into the cytoplasmic fraction.

Table 5

Correlation Analysis of Relationships of Parameters of Activity and Distribution of Lysosomal Cysteine Proteinases of Kidney Tissue with Markers of Oxidative Damage to Proteins

	S_{tot}		$S_{ADNPH,\ sum}$	
	R	**p**	**R**	**p**
Cathepsin B TA	-0,30	0,168	-0,25	0,255
Cathepsin L TA	-0,57	0,005	-0,57	0,006
Cathepsin H TA	-0,0006	0,998	-0,11	0,618
Cathepsin B TA	-0,26	0,244	-0,20	0,370
Cathepsin L TA	-0,57	0,005	-0,57	0,006
Cathepsin H TA	0,04	0,865	-0,07	0,749
Cathepsin B NSA	-0,72	0,0002	-0,74	0,00008
Cathepsin L NSA	-0,58	0,005	-0,65	0,001
Cathepsin H NSA	-0,57	0,01	-0,65	0,001
Cathepsin B NSA%	-0,55	0,008	-0,61	0,002
Cathepsin L NSA%	-0,48	0,023	-0,56	0,006
Cathepsin H NSA%	-0,58	0,005	-0,59	0,004
AP sum NSA%	-0,83	0,000002	-0,84	0,000001
APtsen NSA%	-0,69	0,0004	-0,71	0,0002
APtst HCA%	-0,87	0,0000001	-0,85	0,0000004

It should be noted that reduction in the activity of cathepsins in oxidative stress in hyperhomocysteinemia and correction of the parameters by arginine, found in the studied models, may be explained not only by reduction in their synthesis due to oxidative dama-

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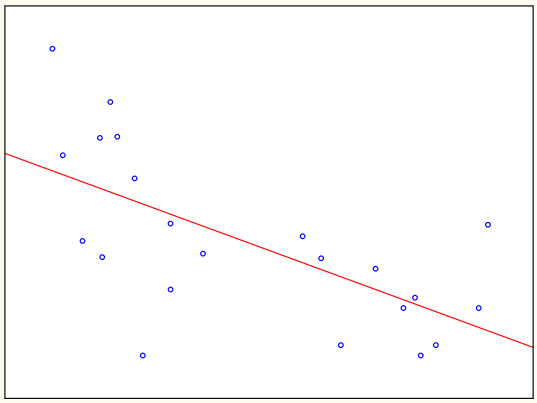
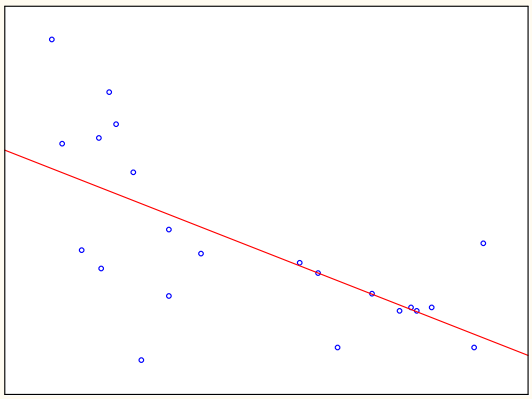
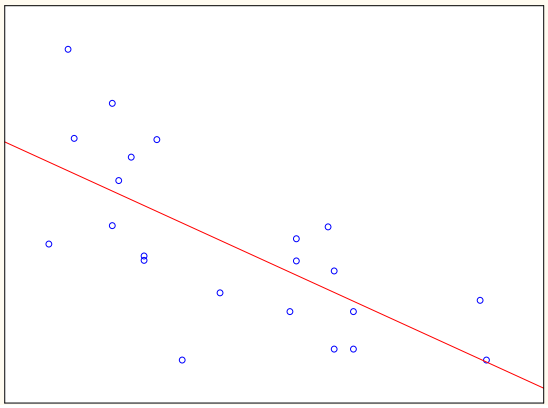


NSA of cathepsin H / S_{tot}
(p=0,001)

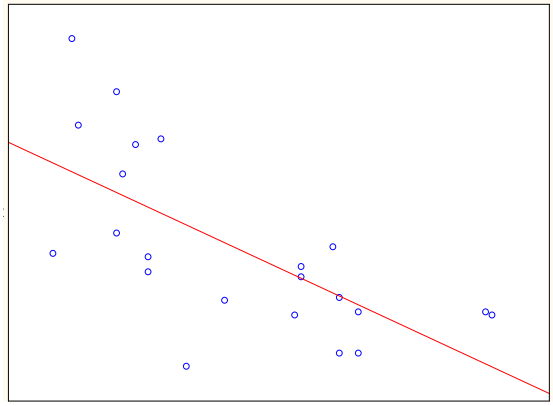


NSA of cathepsin H / $S_{ADNPH, sum}$
(p=0,001)

Fig. 1. Correlation relationships between nonsedimentary activity of cathepsin H of liver and parameters of oxidative carbonylation of proteins

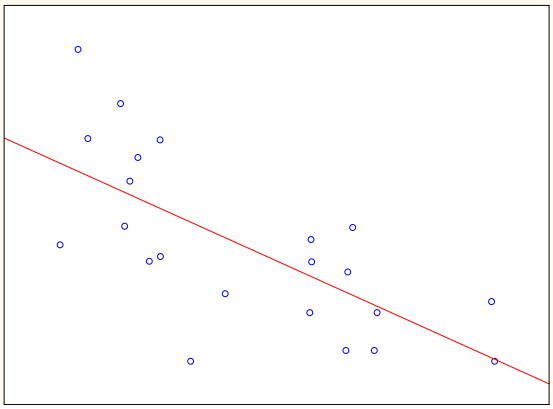


SA of cathepsin H / S_{tot}
(p=0,00005)

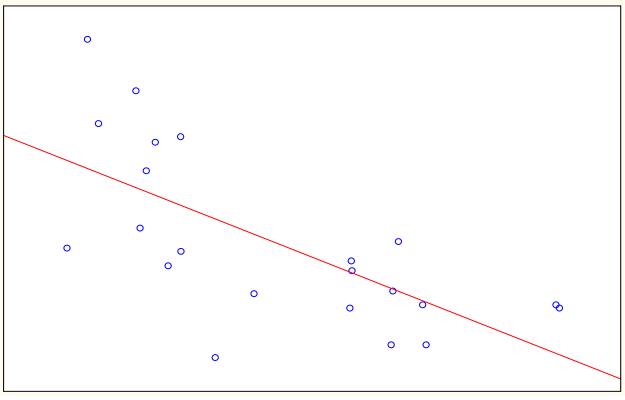


SA of cathepsin H / $S_{ADNPH, sum}$
(p=0,00005)

Fig. 2. Correlation relationships between sedimentary activity of cathepsin H of liver and parameters of oxidative carbonylation of proteins



TA of cathepsin H / S_{tot}
(p=0,00008)



TA of cathepsin H / $S_{ADNPH, sum}$
(p=0,00008)

Fig. 3. Correlation relationships between total activity of cathepsin H of liver and parameters of oxidative carbonylation of proteins

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ge to nucleic acids [15] or by reduction in the activity of enzymes in their direct oxidative modification [16], but also by change in the proportion of active and proenzyme forms. To check the last thesis we evaluated the coefficient of the autocatalytic effect (K_{aca}) of the studied cathepsins (Table 6) because the autocatalytic processing for them is given as a mechanism of effective conversion of zymogen to the active form of the enzyme [17-19].

It was found that numerous changes in the activity of cathepsins in renal tissue in oxidative stress in experimental hyperhomocysteiemia were not accompanied by changes in the coefficient of autocatalytic action which is the reason to believe that the propor-

Table 6

Values of Coefficient of Autocatalytic Action of Cathepsins B, L, H in Experimental and Control Groups (Me [Q_1; Q_3])

Parameter	Group/Organ		
	Control 1		
	Liver	Kidney	Lung
Cathepsin B, NSA	0,18 [0,12;0,40]	1,01 [0,79;1,17]	0,49 [0,49;0,97]
Cathepsin B, SA	0,47 [0,14;0,71]	0,97 [0,68;1,09]	0,76 [0,67;0,97]
Cathepsin L, NSA	0,80 [0,74;0,83]	0,87 [0,76;0,95]	0,62 [0,31;0,86]
Cathepsin L, SA	0,83 [0,72;1,28]	0,95 [0,88;1,02]	0,84 [0,66;1,14]
Cathepsin H, NSA	0,63 [0,35;0,79]	0,87 [0,81;1,19]	0,49 [0,37;0,54]
Cathepsin H, SA	0,60 [0,40;0,80]	0,89 [0,86;0,94]	0,25 [0,18;0,47]
	Methionine		
	Liver	Kidney	Lung
Cathepsin B, NSA	0,73 [0,46;0,98]*, p_1=0,03	0,95 [0,77;1,12]	0,81 [0,61;0,98]
Cathepsin B, SA	0,68 [0,58;0,91]	0,81 [0,63;0,96]	0,49 [0,33;1,22]
Cathepsin L, NSA	1,04 [0,56;1,12]	0,90 [0,65;1,53]	1,16 [0,85;3,99]*, p_1=0,03
Cathepsin L, SA	0,67 [0,54;0,81]	0,90 [0,82;1,08]	1,03 [0,94;1,16]
Cathepsin H, NSA	0,49 [0,44;0,6]	0,89 [0,82;0,95]	0,70 [0,60;0,78]*, p_1=0,02
Cathepsin H, SA	0,37 [0,30;0,61]	0,91 [0,83;0,95]	0,39 [0,31;0,49]
	Control 2		
	Liver	Kidney	Lung
Cathepsin B, NSA	1,20 [0,82;1,47]	1,42 [1,36;1,60]	0,35 [0,32;0,37]
Cathepsin B, SA	1,36 [1,15;1,75]	1,47 [0,74;3,78]	1,18 [0,88;1,22]
Cathepsin L, NSA	0,81 [0,71;0,83]	1,02 [0,94;1,26]	0,58 [0,53;0,65]
Cathepsin L, SA	0,75 [0,62;0,85]	0,96 [0,76;1,06]	0,77 [0,69;1,10]
Cathepsin H, NSA	0,67 [0,52;0,77]	1,19 [1,06;1,33]	0,62 [0,55;0,63]
Cathepsin H, SA	0,44 [0,35;0,53]	0,80 [0,72;0,97]	0,38 [0,35;0,41]
	Methionine+Arginine		
	Liver	Kidney	Lung
Cathepsin B, NSA	1,45[1,26;1,61]#, p_2=0,02	0,84[0,81;1,02]*, p_1=0,005	1,2[0,98;2,2]*, p_1=0,02
Cathepsin B, SA	0,69[0,50;0,82]*, p_1=0,005	1,44[1,08;1,62]	0,90[0,84;1,02]
Cathepsin L, NSA	0,60[0,58;0,67]	0,87[0,86;0,89]*, p_1=0,02	0,31[0,08;0,59]#, p_2=0,008
Cathepsin L, SA	0,61[0,55;0,72]	0,8[0,77;1,09]	0,89[0,82;1,08]
Cathepsin H, NSA	0,27[0,25;0,29]*#, p_1=0,008, p_2=0,005	0,99[0,98;1,03]*, p_1=0,05	0,48[0,33;0,6]#, p_2=0,05
Cathepsin H, SA	0,25[0,20;0,36]	0,62[0,50;0,81]	0,33[0,33;0,34]

Note: *p_1 – statistically significant differences from the respective control group, #, p_2 – statistically significant differences from the group receiving methionine

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tion of active and proenzyme forms do not play any role in the given model. Here, use of arginine in experimental hyperhomocysteiemia led to a statistically significant reduction in K_{aca} in the cytoplasmic fraction in comparison with control. Such changes may indicate existence of the most part of proteinases in active form which to some extent confirms the earlier suggestion about a probable role of cathepsins released from lysosomes, in utilization of proteins with oxidative damage as a probable mechanism of correction of changes in experimental hyperhomocysteinemia by arginine.

However, in hepatic tissue in hyperhomocysteinemia a statistically significant increase in K_{aca} was obtained for nonsedimentary activity of cathepsin B which permits to suggest that the above described increase in its activity in the cytoplasmic fraction may be caused by retardation of maturation of zymogens: for other parameters no statistically significant differences were obtained. It is interesting that application of arginine in experimental hyperhomocysteinemia leads to further reduction in the activity of cathepsin B in the cytoplasmic fraction and also leads to further considerable increase in K_{aca}. Earlier found changes in the activity of cathepsins L and H in hepatic tissue for the studied models were not accompanied by statistically significant changes in Kaca which does not give the ground to suggest the contribution of changes in the proportion of proenzyme and active forms into the detected shifts. However, introduction of arginine with the underlying experimental hyperhomocysteinemia led to a statistically significant reduction in Kaca in nonsedimentary fraction in comparison with both the group with methionine and control group which shows that the detected partial correction of reduced activity of the given enzyme in the cytoplasmic function in the oxidative stress may be associated with increase in the share of active forms.

An interesting finding may be detection of changes of Kaca for lung tissue. Although, according to the earlier presented data, this tissue does not show any changes in the parameters of oxidative damage to proteins and in the activity of cathepsins, it appeared that development of experimental hyperhomocysteinemia led to a statistically significant increase in Kaca for cathepsins L and H in the nonsedimentary fraction, and introduction of arginine returned the parameters to the control values. Thus, in experimental hyperhomocysteinemia the given tissue was characterized by increase in the share of inactive forms without statistically significant reduction in the activity which can be interpreted as a compensatory increase in synthesis and release into the cytoplasm of the mentioned cathepsins which finally created resistance to increase in the level of proteins with oxidative damage.

Rather similar changes in compartmentalization of the studied cathepsins in oxidative stress caused by experimental hyperhomocysteinemia and in particular in its correction by arginine, give the ground to suggest the existence of a universal mechanism of redistribution of enzymes from lysosomes to the cytoplasm. The most probable cause here may be the general increase in the permeability of the lysosomal membrane in oxidative stress [20]. The given suggestion is confirmed by the results of evaluation of the share of nonsedimentary fraction of a marker lysosomal enzyme – acid phosphatase (Table 7): introduction of arginine induced a statistically significant increase in the parameters (as compared to the group with isolated introduction of methionine) not only for renal tissue where values in hyperhomocysteinemia showed a statistically significant reduction, but also for the hepatic tissue that initially did not show any statistically significant differences. Here, the lung tissue was the only tissue in experimental hyperhomocysteinemia that showed a considerable increase in the total activity of acid phosphatase in the group receiving methionine which may result from a compensatory increase in the amount of lysosomes in this tissue and correlates with the earlier made suggestion about a compensatory increase in synthesis of cathepsins. This may probably be the process that created resistance of lung tissue to oxidative damage of proteins with the

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Table 7

Changes in Activity and Compartmentalization of Fractions of Acid Phosphatase in Experimental and Control Groups (Me [Q_1; Q_3])

Parameter	Group/Organ		
	Control 1		
	Liver	Kidney	Lung
AP_{sum}, ncat/g of protein	310,2 [303,3;333,9]	663,4 [629,8;859,3]	514,6 [424,9;607,9]
NSA% AP_{sum}	6,2 [5,7;6,9]	4,2 [3,4;5,0]	2,0 [2,0;2,6]
NSA% AP_{tsen}	3,0 [2,7;3,7]	2,8 [2,0;3,5]	2,8 [2,0;3,5]
NSA% AP_{tst}	10,4 [9,0;11,6]	5,8 [3,9;7,0]	2,3 [1,8;2,7]
	Methionine		
	Liver	Kidney	Lung
AP_{sum}, ncat/g of protein	313,5 [298,5;362,6]	742,8 [643,8;862,00]	933,0[802,6;1148,8]*, p_1=0,001
NSA% AP_{sum}	6,0 [5,2;6,3]	1,8 [1,6;2,0]*, p_1=0,003	1,4 [1,0;1,6]*, p_1=0,005
NSA% AP_{tsen}	3,9 [2,4;5,1]	1,4 [0,9;1,7]*, p_1=0,007	0,9 [0,2;1,1]*, p_1=0,005
NSA% AP_{tst}	7,6 [6,0;9,6]	2,3 [1,6;2,5]*, p_1=0,004	1,9 [1,1;2,6]
	Control 2		
	Liver	Kidney	Lung
AP_{sum}, ncat/g of protein	204,7 [187,8;247,3]	591,1 [509,9;678,4]	523,9 [432,6;643,5]
NSA% AP_{sum}	5,2 [4,6;7,1]	3,9 [3,5;4,3]	1,9 [1,7;2,5]
NSA% AP_{tsen}	4,4 [3,7;5,2]	3,7 [2,9;4,0]	1,9 [1,4;4,6]
NSA% AP_{tst}	5,3 [5,1;7,7]	4,1 [3,4;4,8]	2,1 [1,8;2,2]
	Methionine+Arginine		
	Liver	Kidney	Lung
AP_{sum}, ncat/g of protein	309,9[271,5;328,1]*, p_1=0,02	382,2[365,3;397,9]*#, p_1=0,005, p_2=0,002	588,7[571,9;619,1]#, p_2=0,002
NSA% AP_{sum}	7,0 [6,5;8,3]#, p_2=0,03	4,7 [4,5;4,8]*#, p_1=0,02, p_2=0,002	1,6 [1,5;1,7]
NSA% AP_{tsen}	7,6 [7,5;8,5]*#, p_1=0,008, p_2=0,03	3,1 [2,9;3,2]#, p_2=0,002	2,4 [1,8;3,0]#, p_2=0,004
NSA% AP_{tst}	7,0 [5,9;8,3]	6,6 [5,9;7,2]*#, p_1=0,005, p_2=0,002	1,3 [1,0;1,5]*, p_1=0,008

Note: *p_1 – statistically significant differences from the respective control group, #, p_2 – statistically significant differences from the group receiving methionine

total absence of visible changes in the activity of cathepsins. Here, in experimental hyperhomocysteinemia, the share of the cytoplasmic activity of the total and tartrate-sensitive acid phosphatase in lung tissue decreased, and introduction of arginine caused increase in NSA% for tartrate-sensitive fraction.

Conclusions

1. Arginine at a dose 500 mg/kg introduced within 10 days completely corrects increase in the products of oxidative carbonylation of proteins that develops against the background experimental hyperhomocysteinemia.

2. Action of arginine consists in increase in the activity of cathepsins B, L, H in the cytoplasmic fraction of the liver and kidney, reduced in isolated hyperhomocysteinemia, due to intracellular redistribution of enzymes.

3. Arginine causes changes in the compartmentalization of lysosomal cysteine proteinases through nonselective increase in the permeability of lysosomal membrane.

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4. Inverse correlations were found between concentrations of products of oxidative modifications of proteins and activity of cathepsins in cytoplasmic (nonsedimentary) fraction and the share of their nonsedimentary activity, that permits to suggest a contribution of changes in compartmentalization of lysosomal cysteine proteinases into compensation of oxidative stress in experimental hyperhomocysteinemia under action of arginine.

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