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Cathepsins B, L and H splenocytes as the secondary antioxidant systems in the conditions of carbonyl stress

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Abstract: In the conditions of synthesis of nitrogen oxide deficit` modelling there is a growth of carbonyl derived proteins due to basic aldehyde- and keton-dinitrophenylhydrazones. After adding L-NAME into the incubation medium there appears aggravation of the carbonyl stress as the result of growth of oxidative stress secondary markers content and exhausting of reserve-adaptative resource. Activation of cathepsins L and H splenocytes takes place in response to oxidative modified proteins elaboration which is proved by our positive correlative conjunction.

Keywords: Carbonyl Derived Proteins, Cathepsins, Oxidative Stress, L-NAME

1. Introduction

Excessive production of oxygen and nitrogen active forms leads to development of the number of morbid conditions and taxis, marked consequently oxidative and nitrosative stresses [4, 5]. In its turn accumulation of the active carbonyl compounds (aldehydes and ketones) causes structural and functional alterations of protein molecules leading to development of carbonyl stress [6].

Proteolytic systems are able to delete harmed proteins, that`s why they are considered to be secondary antioxidant systems. Modified proteins destroyed by proteasome or protease to peptides and/or amino acids can be the resource of synthesis of the new protein, needful to cell. That`s why oxidation of proteins is the essential part of protein metabolism in vivo with the participation of the histologic proteinase.

2. Materials and Methods

The research was conducted on conventional pubescent masculine rats brood Wistar. For getting biological cell bulk animals were anaesthetized and dehematized for spleen exhaustion. Purified tissue was put into glass tissue grinder containing complete nutrient in the ratio 1/10, and comminuted by manual pestle rotation. Then derived suspended cells were incubating in complete nutrient with addition of 5 mM L-NAME for 24 hours at the temperature of 37°C. Cells liability was evaluated before and after the incubation with the tube method in Gorjaev's chamber with the use of 0,2% trypan blue in physiological solution as the colouring agent.

Activity of the cathepsins B, L and H was examined by the spectrofluorimetric method of Barret & Kirschke [1]. The definition of carbonyl derived proteins of R.L. Levine [3] was used for evaluation of oxidative proteins` modification. We built up spectrum of carbonyl derived proteins` absorption and calculated area under the curve. For primary markers content evaluation we counted the sum of aldehyde-dinitrophenylhydrazones, for evaluation of secondary markers – sum of keton-dinitrophenylhydrazones and this was correlated to the total content (S total) of carbonyl derived proteins.

Reserve-adaptative resource was evaluated through calculating ratio of area under the curve of carbonyl derived proteins in case of the spontaneous oxidation of proteins to the induced oxidation according to Fenton reaction with the condition that keton-dinitrophenylhydrazones` indicator and aldehyde-dinitrophenylhydrazones` indicator were equal 100%.

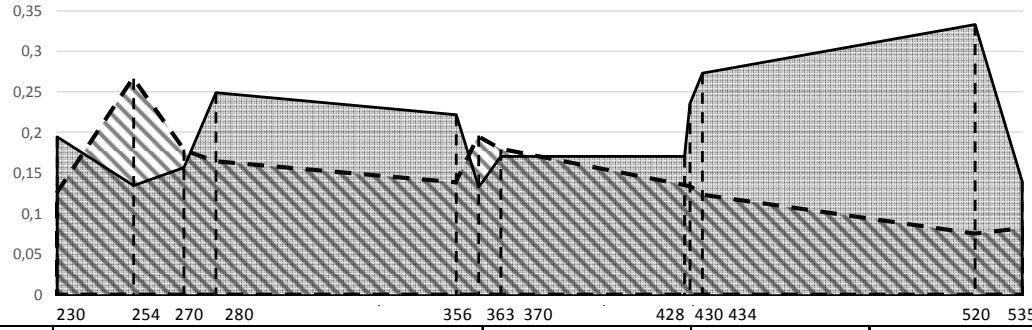
3. Results and Discussion

Over the last years endogen produced nitrogen oxide

considers as one of the oxidative stress agents. At the present time NOS inhibitors derived from the substrate of NO synthesis – L-arginine are the most extensively studied. The inhibition mechanism is based on competitive interaction of substrate and inhibitor for the zymophore. Important step in pathophysiology of NO was the discovery of natural endogen competitive inhibitor NOS – asymmetric dimethylarginine (ADMA), the presence of which leads to distortion of metabolism and membrane transport of L-arginine and effects NO biosynthesis decrease. ADMA-cloning exogenous inhibitor is Nω- nitro-L-arginine-methyl ether (L-NAME).

The liability of newly educed splenocytes reached 71±4,8%. After incubation the liability of the control group of splenocytes decreased insignificantly and become 68±3,0% and in the experimental group this mark decreased in comparison with newly educed cells and incubated control group and became 49±3,2% (p=0,02). This mark indicates that of by the in vitro nitrogen oxide synthesis deficit model nitrosplenocytes are more subjected to programmed death. Partially oxidized proteins can go through further oxidation with the following development of aggregates accumulating in the cell. In this situation the process of apoptosis can be the defense mechanism which helps to remove unwelcome cells. Lysosomal cysteine proteinases and nitrogen oxide take part in the regulation of apoptosis. The abovementioned hypothesis is coherent with the information from literature and is supported by the findings: by the in vivo decrease of NO metabolites` concentration and increase of cathepsins activity and expansion in the number of carbonyl derived proteins the liability of splenocytes decreases.

Comparing the results of area under the curve of carbonyl derived proteins splenocytes incubated in complete nutrient with addition of 5 mM L-NAME to the amounts of the control group we got the following conclusion – the total area under the curve of modified proteins` absorption grows statistically significant due to statistically significant growth of basic aldehyde-dinitrophenylhydrazones and keton-dinitrophenylhydrazones.



	S aldehyde-dinitrophenylhydrazone of neutral character	S keton-dinitrophenylhydrazone of neutral character	S aldehyde-dinitrophenylhydrazone of basic character	S keton-dinitrophenylhydrazone of basic character	S total
Control	15,8 [15,6; 20,5]	7,4 [4,5; 9,8]	6,5 [4,7; 9,7]	1,1 [0,8; 1,7]	33,4 [25,7; 39,4]
L-Name	25,7 [11,7; 44,9]	10,6 [7,1; 31,8]	23,5 [9,8; 44,9]*	4,7 [2,1; 8,0]*	72,1 [31,3; 129,8]*

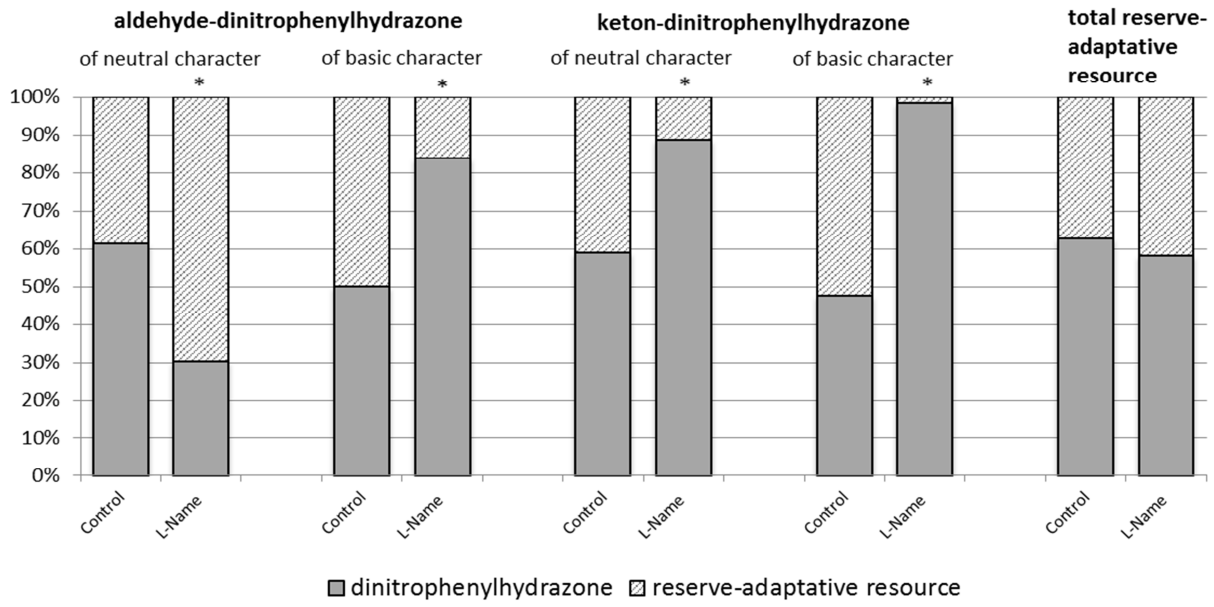
Notice*: statistically significant differences of control fraction (p<0,05).

Picture 1. *Comparative study of modified splenocytes proteins` absorption spectrum of rats under the influence of 5 mM L-NAME in vitro.*

Nitrogen oxide is referred to the systems of non-enzymatic control of reactive oxygen intermediates` rate and that`s why, probably, in the conditions of NO synthesis deficit modeling the reactive oxygen intermediates become the main destructive agent. Moreover, discovered alterations can be caused by decreased activity of the antioxidant system with the disbalance between the processes of generation and deactivation of reactive oxygen intermediates.

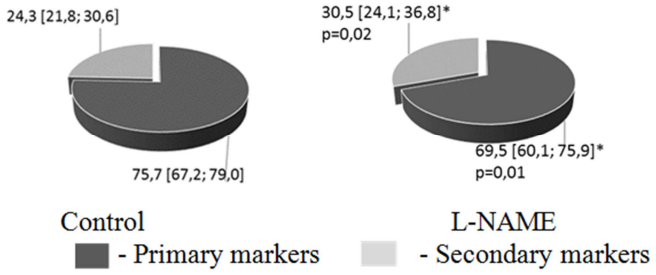
Indirect estimation of abilities of antioxidant proteins` repelling can be realized with the use of reserve-adaptative resource` indicator. In the conditions of rats` splenocytes incubation in complete nutrient with addition of 5 mM L-NAME there was exhausting of reserve-adaptative resource of basic aldehyde-dinitrophenylhydrazones and keton-dinitrophenylhydrazones (p=0,036 and p=0,0036 consequently) and neutral keton-dinitrophenylhydrazones (p=0,042) comparatively to the control results. However, amount of the total reserve-adaptative resource of the experimental group doesn`t differ from the control amount statistically significant, probably, due to growth of reserve-adaptative resource of neutral aldehyde-dinitrophenylhydrazones, p=0,03 (picture).

Therefore we discovered that amount of reserve-adaptative resource in spleen decreases under the influence of L-NAME. This can indicate not only of antioxidant systems exhaustion, but also it can demonstrate availability of protein molecules for damaging action caused by denaturation molecule.



Notice*: statistically significant differences from control group ($p< 0,05$)

Picture 2. *Condition of splenocytes` reserve-adaptative resource in the context of in-vitro NO synthesis deficit modelling.*

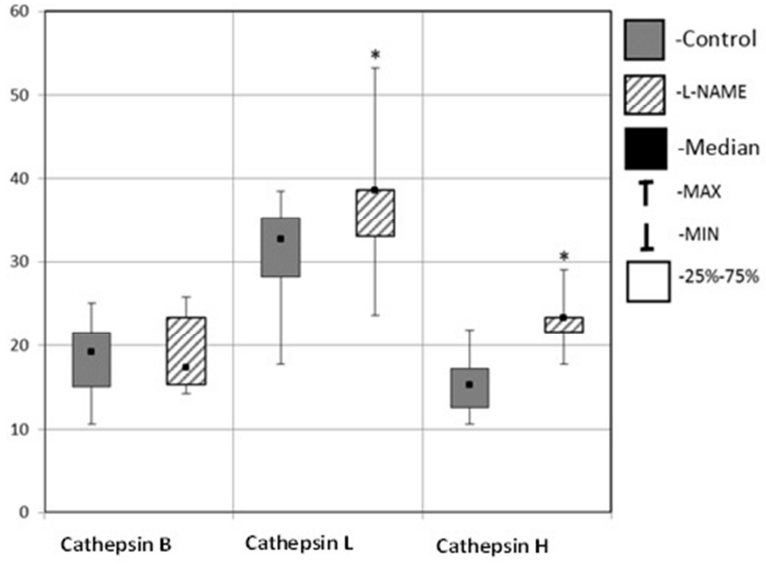


Notice*: statistically significant differences from control group ($p< 0,05$)

Picture 3. *Amount of primary and secondary markers of the oxidative stress in rats` splenocytes in the context of in-vitro NO synthesis deficit modelling, Me (min, max).*

Examination of ratio of primary markers (aldehyde-dinitrophenylhydrazones) and secondary (keton-dinitrophenylhydrazones) markers of oxidative stress gives the opportunity to evaluate phase and potential reversibility of oxidative stress` development. Under the influence of L-NAME in splenocytes and in spleen tissue it is observed that amount of the secondary markers of the oxidative stress grows comparatively to this amount in the control group. That indicates aggravation of the oxidative stress and its transition to the advanced stage.

In the context of in-vitro NO synthesis deficit modelling there is a growth of total cathepsins L and H activity comparatively to the amounts of the control group (picture 4).



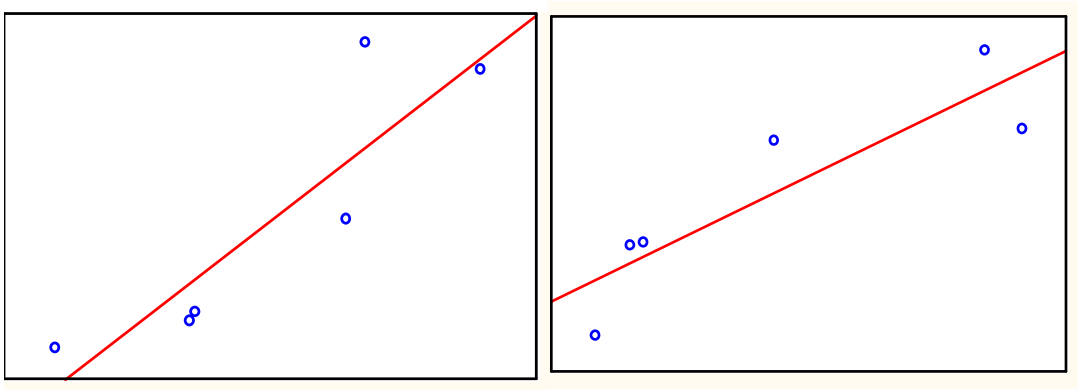
Notice*: statistically significant differences from control group ($p< 0,05$)

Picture 4. *Activity of cathepsins B, L and H splenocytesin the context of in-vitro NO deficit synthesis modelling.*

Endocellular rate of oxidative proteins reflects the balance between process of carbonyl proteins developing and degradation factor caused by proteolytic systems. There is hypothesis that appearance of hydrophobic groups at the surface of the protein molecules is the impulse factor of proteolytic sensibility boost. It is known that proteinases destroying oxidized proteins are more selective to the damaged basic amino-acid residues [2]. In conditions of NO synthesis deficit we discovered growth of basic amino-acid residues.

For evaluation of lysosomal proteolysis contribution to metabolism of modified proteins we examined cathepsins activity in the conditions of metabolites rate alteration during NO synthesis. In conditions in vivo- and in vitro-retardation of NO synthesis the total activity of lysosomal cysteine proteinases (cathepsins B, L and H) grows.

While analyzing findings we elicited direct correlative links between the total area under the curve of oxidative proteins modification and the total activity of cathepsins L and H of the spleen under the influence of L-NAME in conditions in-vitro. This tendency indicates existence of correlation between the oxidative affection of proteins processes and lysosomal cysteine proteolysis in conditions of NO synthesis deficit.



a b

Picture 5. *The correlative links between the total area under the curve of spectrum of carbonyl derived proteins` absorption (S total) and total activity (OA) of cathepsins L (a) and H (b) of the spleen.*

The sum-total of findings enables to consider involvement of lysosomal proteinases into the process of carbonyl derived proteins removal as one of possible mechanisms not only of protein metabolism in tissues but also as a defense mechanism of the secondary antioxidant systems. Cathepsins impede accumulation of oxidative modified proteins and by that prevent their accumulation and development of the oxidation process.

4. Conclusions

1. Inhibition of NO synthesis by the competitive inhibitor N-nitro-L-arginine-methyl ether leads to accumulation of carbonyl derived proteins in the splenocytes with domination of basic dinitrophenylhydrazones.
2. In conditions of NO synthesis deficit there is the activation of lysosomal cysteine proteinases L and H splenocytes.
3. The positive correlative links between the oxidative carbonylation of proteins and activity of lysosomal cysteine proteinases L and H of the spleen indicated in conditions of NO synthesis deficit.

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