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CORRECTION OF NO-INDUCED APOPTOSIS IN JURKAT-CELLS CULTURE WITH THE HELP OF PLAFERON LB

Apoptosis or cell self-liquidation process is the active and organized form of cell death.

Physiological apoptosis is developed during embryogenesis, when auto-reactive lymphocytes are eliminated from peripheral blood, etc. It has mitosis opposite function and is important for tissue size regulation and maintaining of homeostasis in healthy body. Violation of physiological apoptosis may cause carcinogenesis, auto-immune diseases, etc.

Apoptosis inducing stimuli, in certain conditions, can cause even necrosis. So, possibility of development of apoptosis or necrosis is determined by intensity of these processes inducing stimuli.

Resulting from the above, pharmacological regulation of apoptosis plays a significant role.

It is well-known that the mechanism of cell death (apoptosis/necrosis) considerably depends on energy-genesis intensity. Decreasing of the cell energy resources causes development of oxidation stress and initiation of apoptosis. Sharp decrease of energy-genesis and expiration of energy in a cell causes replacement of apoptosis with necrosis. The process of energy-genesis is closely related to intensity of mitochondrial breathing, in regulation of which the NO molecule-manager plays an important role through nitrosification of proteins transmitting electrons of breathing chain.

Resulting from the above, determining certain mechanisms of NO-induced apoptosis development and effectiveness of its correction with the help of Ploferon LB gains theoretical as well as practical significance.

Purpose of the research:

The research aims at determining certain mechanisms of NO-induced apoptosis development and effectiveness of its correction with the help of Ploferon LB in Jurkat-cells culture (mature T-cells transformed by leukemia).

Materials and methods:

The research was carried out on mature T-cells transformed by human leukemia (Jurkat-cells) (DSMA-Deutshe Sammulung von Mikroorganismen und Zellkulturen) (Germany).

For apoptosis modeling we were adding ImM Na Nitroprusside (Naniprus, Sopharma) to cell suspension and were incubating it during 24, 48 and 72 hours. To the part of the cells, together with Na Nitroprusside, we were adding Plaferon LB (in doses of 0,2 µg and 0,4 µg). In the above mentioned expert terms intact Jurkat-cells represented a control group. With the help of the method of cytometry in progress in different periods of the post-incubation

time, we were studying the distribution of Jurkat-cells over cell cycle phases, determining the changes of mitochondrial-membrane potential; with the help of electronic paramagnetic resonance and spin traps we were identifying the content of free nitric oxide (NO), hemic and non-hemic iron nitrolized complexes (HbNO, FeSNO), superoxide (O_2) and lippo peroxide radicals (LOO).

With the help of the method of cytometry in progress in the cell culture, using lipophilic cationic sample 3,3'-dihexiloxacarbocanine iodide – $DiOC_6$, we were determining the changes in mitochondrial-membrane potential.

We were studying distribution of cell cycle phases on the basis of cytometry in progress through the method of painting with propodium iodide.

Results of the research:
After simultaneous incubation of Jurkat-cells together with Na Nitroprusside and Plaferon LB in doses of 0,2 μg and 0,4 μg) during 24, 48 and 72 hours, percentage of high mitochondrial-membrane potential significantly increases and vice versa (Table 1).

Table 1

Cell potential in Jurkat-cells culture is distributing according to the changes of mitochondrial-membrane potential after incubation together with Na Nitroprusside and Plaferon LB

Jurkat cells	Control	Na – Nitroprusside, 24 hours	Na – Nitroprusside, 48 hours	Na – Nitroprusside, 72hours	Na – Nitroprusside, + PLB, 24 hrs, 0,2 μg/ml	Na – Nitroprusside, + PLB, 48 hrs, 0,2 μg/ml	Na – Nitroprusside, + PLB, 72 hrs, 0,2 μg/ml	Na – Nitroprusside, + PLB, 24 hrs, 0,4 μg/ml	Na – Nitroprusside, + PLB, 48 hrs, 0,4 μg/ml	Na – Nitroprusside, + PLB, 72 hrs, 0,4 μg/ml
Total:	100 %	100%	100%	100%	100%	100%	100%	100%	100%	100%
M1	66, %	51.0%	33,9%	13,2%	76,6%	53,0%	37,0%	76,0%	71,0%	69,9%
M2	33, %	49,0%	66,1%	86,8%	23,4%	47,0%	63,0%	24,0%	29,0%	30,1%

M1 – the cells with high mitochondrial-membrane potential (in %)
M2 – the cells with low mitochondrial-membrane potential (in %)

Percentage of the cells with low mitochondrial-membrane potential significantly decreases in comparison with the indicators characteristic for Na Nitroprusside.
In Jurkat-cells culture, when influenced with Na Nitroprusside and Plaferon LB, EPR signals are changing. In this situation in Jurkat-cells culture the content of oxygen and lipids'

reactive matter, degree of nitrolizing of mitochondrial electrons transport proteins (FeSNO, HbNO) is decreasing (or never shows up) and content of free NO (due to low transformation into peroxide nitrite) and intensity of mitochondrial breathing (due to increasing of free radicals EPR signals) is increasing in comparison with the indicator characteristic for Na Nitroprusside (Table 2).

Table 2

Changes of EPR signals in Jurkat-cells culture when influenced with Na Nitroprusside and Plaferon LB

		O_2	NO	LOO	Free Radic.	FeSNO	HbNO
Control	24 hrs	-	10,0±0,5	-	10,0±0,5	-	-
	48 hrs	-	10,0±0,6	-	12,0±0,6	-	-
	72 hrs	-	10,0±0,6	-	11,0±0,6	-	-
Na Natroprusside	24 hrs	3,0±0,6	10,0±0,5	-	11,0±0,6	5,0±0,6	10,0±0,6
	48 hrs	5,0±0,6	10,0±0,7	10,0±0,5	5,0±04*	6,0±0,5	12,0±0,4
	72 hrs	9,0±0,5	9,0±0,8	12,0±0,9	3,0±0,4*	6,0±0,6	11,6±0,6
Na Natroprusside + PLB 0,2 µg/ml	24 hrs	-	10,0±0,5	-	13,0±0,5**	5,0±0,6	13,0±0,8**
	48 hrs	3,1±0,6**	16,0±0,5**	6,0±0,7**	12,0±0,5**	4,0±0,6**	8,0±0,6**
	72 hrs	3,0±0,6**	16,0±0,6	4,7±0,6**	12,0±0,6**	4,0±0,6**	7,0±0,6**
Na Natroprusside + PLB 0,4 µg/ml	24 hrs	-	15,0±0,8	-	12,0±0,5	3,0±0,6**	-
	48 hrs	-	20,0±0,9	-	10,5±0,6**	2,0±0,5**	-
	72 hrs	-	19,0±0,8**	-	10,0±0,6	1,5±0,6	-

* - reliable difference in comparison with corresponding indicators of control series

** - reliable difference in comparison with the corresponding indicators characteristic with Na Nitroprusside

Intensification of mitochondrial breathing is causing strengthening of energy-genesis, which is becoming evident through increasing of mitochondrial-membrane potential, which, in its turn, facilitates apoptosis reduction. The latter has been revealed when studying Jurkat-cells distribution in the cell cycle phases through reduction of the number of apoptosis cells (Table 3). At the same time, as it can be seen from the test results, increasing of number of

mitosis has been detected on the basis of Plaferon LB impact, especially, through 0,4 μg impact which points to intensification of proliferation processes.

Table 3

Percentage of distribution of cells in Jurkat-cells in cell cycle phases after incubation with Na Nitroprusside and Plaferon LB

Jurkat cells	SubGO (Apopt.)	GO/G1	S	G2/M
Control	16,8%	55,2%	13,0%	15,0%
Na Nitroprusside – 48 hrs	43,6%	37,4%	11,5%	7,5%
Na Nitroprusside – 72 hrs	55,9%	27,0%	11,2%	5,95
Na Natroprusside + PLB 0,4 μg/ml 48 hrs	29,5%	39,5%	16,5%	14,5%
Na Natroprusside + PLB 0,4 μg/ml 72 hrs	21,0%	39,0%	21,0%	19,0%

As a result of the electronic-microscopic investigation of Jurkat-cells incubated with Na Nitroprusside and Plaferon LB, as in the previous series of the experiment, diversity of changes is revealed, which is especially exhibited in the conditions of Plaferon LB influenced by 0,4 μg dose after incubation of 72 hrs.

In a part of cells, the plasma membrane is smooth, in-contour, lysis of separate section is seldom (which is frequent only during incubation with Na Nitroprusside), cytoplasm is light, the number of vacuoles is high, the number of ribosomes and polysomes is low, mitochondrion has the form of empty oval bodies. Nucleus, basically, is repeating the form of the cell. Several nuclei reveal marginalization, condensation of chromatin close to walls and small nucleoli.

Conclusion: In Jurkat-cell, Plaferon LB causes culture reduction of intensity of No-induced oxidation stress, increase of mitochondrial-membrane potential, which, in its turn, provides reduction of apoptosis intensity, increase of mitosis and intensification of cell proliferation.

Practical Recommendations:

1. Considering the significant role of pro-oxidation processes in the apoptosis mechanism, we recommend application of the preparations of anti-oxidant nature with the purpose of apoptosis suppression.

2. Anti-apoptosis activity of Plaferon LB on Jurkat-cell culture, revealed during the investigation, enables us to recommend, with the purpose of prevention of T-lymphocyte apoptosis, application of this preparation in the process of treatment of various infectious

diseases, neurodegenerative pathologies, ischemic diseases, AIDS, helicobacter pylor induced gastritis and stomach ulcer.

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