

## The changes in mitochondria respiration chain during NO-induced apoptosis and influence preparation plaferon LB in Jurkat cell culture

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### ABSTRACT

The aim of our study was to establish the changes in mitochondria respiration chain during NO-induced apoptosis and influence preparation Plaferon LB in Jurkat cell culture. During incubation with natrium nitropruside (SNP) (24 and 48 hours) in Jurkat cells mitochondrial respiratory chain it was observed nitrosilation of electron transport proteins with formation of hemic (HbNO) and nonhemic (FeSNO) complexes, rising of mitochondrial respiration intensity, containing of free nitric oxide and intensification of superoxide- and lipoperoxide radicals generation. Under influence of Plaferon LB the concentration of nitric oxide and intensity of mitochondrial respiration in Jurkat cells restore, the intensity of oxidative stress decreased and revealed by reduction of superoxide and lipoperoxide radicals content.

**KEYWORDS:** mitochondria respiration chain, nitric oxide, apoptosis, plaferon LB, Jurkat cell

In apoptosis initiation taken part intra- and intracellular stimulus. To intracellular stimulus belongs damage of DNA, development of cellular metabolic disbalance, in which mitochondrial disorders play important role. Deliverance of mitochondrial pro-apoptotic proteins induces activation of kaspasa-8 and kaspasa 3 and initiation of apoptotic cascade. In this case mitochondria appear functional but not inducing system and play a direct effectors role in apoptosis initiation. Mitochondria-dependent apoptosis develops in T-cells Jurkat and CEM lines [1,2,3].

In mitochondria-induced apoptosis mitochondria undergo functional changes, with decrease of membrane potential ( $\Delta\psi$ ) [4,5,6]. Mitochondria-dependent apoptosis development takes place during depletion of restore glutamate NAD(p)H, ATP and ADP, accumulation of oxygen reactive species, rising of cytoplasm  $Ca^{2+}$ , influence of ceramid, NO and other proapoptotic stimulus.

The aim of our study was to establish the changes in mitochondria respiration chain during NO-induced apoptosis and influence of preparation Plaferon LB in Jurkat cell culture.

### MATERIALS AND METHODS

Human adult T cell leukemia (Jurkat cells) (DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen) were grown by using biological active medium RPMI 1640 (GIBCO), inactivated embryonic calf serum, L-glutamine (4 mM), penicillin (100 U/ml), streptomycin (100 U/ml) at 37°C in humidified atmosphere containing 5% CO<sub>2</sub>. Experiments were carried at concentration of 0,3-0,6 x 10<sup>6</sup> cells in 1 ml area.

In a model of natrium nitroprusid (SNP) induced apoptosis SNP (Naniprus, Sopharma) was added in cells culture in dose 1mM and cells were incubated for 24 and 48 hours. In part of cells the SNP was added simultaneously with plaferon LB (PLB) by dose  $\mu$ M/ml. After incubation the EPR spectra of mitochondrial respiratory chain free radicals of ubisemiquinons and flavoproteides electron transport protein, hemic (HbNO) and nonhemic (FeSNO) complexes, superoxidradicals ( $O_2^-$ ), lipoperoxides (LOO $\cdot$ ), and signals were registered. For detection of superoxidradicals ( $O_2^-$ ), lipoperoxides (LOO $\cdot$ ) and free nitric oxide (NO) EPR signals the spin-traps 5,5 dimethyl-1-pirolin-IV-oxide (DMPO),  $\alpha$ -phenil-

tert-butilnitron (PBN) and Na dietidithiocarbamate (DETC) (SIGMA) were used. The EPR spectra were registered on the EPR spectrometry RE-1307 (Russia).

### RESULTS AND DISCUSSION

In the Tab.1 it is shown changes of EPR signals intensity in Jurkat cells culture, incubated with SNP. As it escape from obtained data, in intact Jurkat cells EPR spectra the low intensity EPR signals of free nitric oxide (NO) and mitochondria respiratory chain ubisemiquinons and flavoproteides free radicals signals were detected. After 24 hours incubation with SNP, in Jurkat cells culture EPR spectra spin-trapped NO and free radical signals' intensity was not changed compare to the control level. The superoxidradicals ( $O_2^-$ ), hemic (HbNO) and nonhemic (FeSNO) iron nitrosil complexes and signal it was revealed. After 48 hours incubation with SNP, in Jurkat cells culture EPR spectra spin-trapped NO and free radical signals' intensity was not changed compare to the control level, but the EPR signal intensity of free radicals was decreased by 50% compare to control level. At this time in Jurkat cells EPR spectra it wasn't revealed superoxides ( $O_2^-$ ), but were appeared lipoperoxides (LOO $\cdot$ ), HbNO and FeSNO complexes EPR signals.

During simultaneous incubation with SNP and Plaferon LB in Jurkat cells culture the EPR signal intensity of NO increased by 50%, the free radicals EPR signal intensity is not changes compare to the SNP-distinctive level. In this experimental series in Jurkat cells EPR spectra was not revealed ( $O_2^-$ ), (LOO $\cdot$ ) and HbNO- complexes EPR signals, but the FeSNO signal intensity reduced greatly. After 48 hours continued incubation with SNP and Plaferon LB in Jurkat cell culture EPR spectra the free nitric oxide EPR signal intensity yet more increases and amounts to composes the 200% of control level. At this time in Jurkat cells EPR spectra it was not revealed superoxidradicales ( $O_2^-$ ), lipoperoxides (LOO $\cdot$ ) and HbNO-complexes signals. The free radicals EPR signal intensity remains at the control level, appears a FeSNO-complexes EPR signal with small intensity.

On the basis of obtained data analysis we can conclude that during the incubation of Jurkat cells culture with SNP the free NO concentration increases greatly.

As it is known, physiological concentrations of NO inhibit cytochrome c oxidase (complex IV) in a reversible manner in competition with oxygen. This could represent a

physiological strategy to reduce  $O_2^-$  consumption. However, long-term exposure to NO can irreversibly inhibit mitochondrial complex I, probably by S-nitrosylation of critical thiols in the enzyme complex. This revealed in our experiments by EPR signal of nitrosil complexes of hemic (HbNO) and nonhemic (FeSNO) iron. Formation of these complexes promotes inhibition of mitochondria electron transport chain and induces development of oxidative stress, manifested by EPR signal of superoxideradicals ( $O_2^-$ ). It is not elided formation of peroxinitrite in condition of excess NO and  $O_2^-$ . After 48 hours of incubation in Jurkat cells culture EPR spectra the high-reactive superoxideradicals ( $O_2^-$ ) were not detected, but it was revealed the lipoperoxids (LOO·) EPR signal, which indicates on the intensification of lipid peroxidation. Decrease of free radical EPR signal intensity indicates on the reduction activity of the mitochondrial electron transport and ATP production of, promoted by inhibition of hemic (cytochrome c oxidase)

and nonhemic (NADH-dehydrogenase) electron transport proteins.

The rice intensity of spin-trapped NO EPR signal during incubation of Jurkat cells with SNP and Plaferon LB (24 hours) indicates on the release of produced by SNP excess NO from mitochondrial transport proteins, which contributes to normalization of mitochondrial electron transport activity (increase of free radical EPR signal to normal level), reduction of oxidative stress (disappearing of spin trapped  $O_2^-$  and LOO· EPR signals). After 48 hares of joint incubation of Jurkat cells with SNP and Plaferon LB the intensity of free NO highly increase, possibly by his reason of prevention of its transformation in peroxinitrite. So based on the result of our experiments we may conclude, that Plaferon LB provides protection of Jurkat cells mitochondria transport proteins from nitrosilation by excess NO and exists normalization of mitochondrial respiration and reduction of oxidative stress and therefore prevents cells from apoptosis.

		$O_2^-$	NO	LOO·	Free radicals	FeSNO	HbNO
Control	24 hour	-	10	-	10	-	-
	48 hour	-	10	-	12	-	-
SNP	24 hour	9	10	-	11	5	10
	48 hour	-	10	10	5	6	12
SNP+PLB	24 hour	-	15	-	12	3	-
	48 hour	-	20	-	10	2	-

**Tab.1** Changes of EPR signals intensity in Jurkat cells culture.

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## Изменения в дыхательной цепи митохондрий при NO-индуцированном апоптозе и воздействии плаферона ЛБ в клетках Jurkat

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### РЕЗЮМЕ

Целью работы являлось исследование изменений в дыхательной цепи митохондрий при NO-индуцированном апоптозе и результатов воздействия плаферона ЛБ (ПЛБ) на клетках Jurkat. Установлено, что во время инкубации с нитропруссидом натрия (в течении 24 и 48 часов) в дыхательной цепи митохондрий клеток Jurkat наблюдается нитрозилирование электронно-транспортных белков с образованием комплексов FeSNO и HbNO, снижение интенсивности митохондриального дыхания и содержания свободного оксида азота в клетках, интенсификация генерирования супероксидрадикалов и липопероксидов в клетках. Под действием ПЛБ восстанавливается содержание свободного NO и интенсивность митохондриального дыхания в клетках Jurkat; снижается интенсивность окислительного стресса, что проявляется уменьшением содержания супероксид- и липопероксидрадикалов в клетках Jurkat.

**Ключевые слова:** дыхательная цепь митохондрии, оксид азота, апоптоз, плаферон ЛБ, клетки Jurkat