

## Influence of plaferon LB on LPS stimulated free radicals' generation in Jurkat cell culture

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### ABSTRACT

The free radical NO has many physiological functions. It has been shown that NO reacts with the cellular components, switches them from physiological to a pathophysiological stages and by this way involves in many diseases. From the above-mentioned the development of new drugs which selectively modulates NO activity is of great importance in modern medicine. We studied the influence of plaferon LB on LPS induced free radical generation in Jurkat cells culture. With EPR method we revealed basic EPR signal of spin-trapped NO in Jurkat cells culture. We show that stimulation by LPS promotes generation of nitric oxide, superoxide- and peroxyradicals in this culture. Decreasing of this free radicals EPR signals intensity by influence of plaferon LB indicates, that this preparation affects on NF-kB' stability and by this way regulates proinflammatory genes expression and cells reactions on mitogens.

**KEYWORDS:** *plaferon LB, free radicals, Jurkat cells*

The simple chemical compound, nitric oxide, is produced persistently in humans' and animals' organisms through the fermentative oxidation of L-arginin (Nathan C., 1992).

The free radical NO has many physiological functions. It works as endothelium-derived relaxing factor (EDRF), inhibits platelet coagulation, serves as the reverse messenger for neuronal transmission and plays an important role in immunity. It can also act as an important regulator of general cellular processes such as gene expression, tissue respiration, oxidative phosphorylation and etc. (Bredt D.S. and Snyder S.H., 1994., Brenman J.E. and Bredt D.S., 1997).

NO is derived from guanidino group of L-arginine in a reaction, catalyzed by a family of NO-synthases (NOS), which consists of neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial (eNOS). nNOS and eNOS are constitutively synthesized in cells and regulate normal homeostatic function of vascular and central nervous systems. Inducible NO-synthase (iNOS) is synthesized after stimulation by microbial endotoxins, cytokines and oxygen stress and the high amount of NO produced by it primarily regulates immune defence of organism.

In addition, purified nNOS and eNOS catalyze superoxide ( $O_2^-$ ) formation (Wang W. et al 2000). Recently iNOS was also found to produce  $O_2^-$  as well as NO, and it was shown that these oxidants could contribute to the antibacterial activity of macrophages (Xia Y. Zweier J.L., 1997).

Character of NO activity depends on type of the target cell, the local concentration and the relative amount of other reactive species. Physiologic concentration of NO inhibits cytochrome c oxidase in a reverse manner together with oxygen. However, long term exposure to NO can irreversibly inhibit mitochondrial complex 1, probably by S-nitrosylation of critical thiols in the enzyme complex (Lamas S et al, 1998). Double-faced character of NO is also illustrated by its ability to either protect cells from apoptosis or cause apoptotic cell death. It has been shown that NO reacts with the cellular components, switches them from physiological to a pathophysiological stages and by this way involves in many diseases. From the above-mentioned the development of new drugs which selectively modulates NO activity is of great importance in modern medicine. At this point of view the new discovered drug plaferon LB, created at the Institute

of Medical Biotechnology of Georgian Academy of Sciences by Prof. V. Bachutashvili is of great interest (USA Patent N WO 02/12444 A2). After prosperous experimental and clinical approbation plaferon LB was registered by Georgian Health Care as immunomodulatory drug and has been successfully applied in clinics for the last 10 years. In addition of immunomodulatory action plaferon LB has antioxidant and antiapoptotic properties (Mitagvaria N., et al, 2001, Bakhutashvili A.V., et al., 2000)

### MATERIALS AND METHODS

Human adult T cell leukemia (Jurkat cells) were grown in suspension using a biological stirring platform in RPMI medium 1640 (GIBCO) supplemented with 10% heat-inactivated fetal calf serum, L-glutamine (4 mM), penicillin (100 units/ml), streptomycin (100 µg/ml), and penicilin (100 µg/ml) at 37°C in humidified atmosphere containing 5% CO<sub>2</sub>. Experiments were conducted using cells in long phase growth at a concentration of 0,3-0,6 x 10<sup>6</sup> per ml.

For mitogen stimulation 2,5 µM lipopolysaccharides (LPS, Sigma) was added to each well containing 1ml cell suspension. To part of the cells plaferon LB was added by dose 0,064 µg. After that cells have been placed in sterile test-tubes and incubated at the 37°C in 5% CO<sub>2</sub> humidified environment for 18 hours.

For the determination of superoxide, nitric oxide and peroxil radicals the EPR measurements were recorded using an EPR spectrometer RE 1307 (Russia) operating at 9.77 GHz with a modulation frequency of 50 kHz and a TM110 cavity. Free nitric oxide spin trapping experiments were performed with spin trap Diethyldithiocarbamic acid Sodium Salt (DETC). Jurkat cells in suspension 5x10<sup>6</sup> cells/ml pre-treated with LPS and plaferon LB were incubated with 0.34 mg/ml DETC for 10 minutes. The cells were then immediately transferred in liquid nitrogen and the EPR spectra recorded. At the liquid nitrogen temperature microwave power [10].

Superoxide radicals spin trapping experiments were performed with spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (56.6 mg/ml). Jurkat cells in suspension 5 x 10<sup>6</sup> cells/ml pre-treated with LPS and plaferon LB were incubated with DMPO for 10 minutes. Peroxil radicals spin trapping were performed with spin trap  $\alpha$ -phenyl-tert-butylhinitrone (PBN) (SIGMA) Jurkat cells in suspension 5 x 10<sup>6</sup> cells/ml pre-treated with LPS and plaferon LB were

incubated with PBN for 10 minutes. The EPR spectra of spin trapped Reactive oxygen and lipids species were recorded at the room temperature and microwave power 20 mVt.

### RESULTS AND DISCUSSION

In the table there are shown data of the LPS-induced nitric oxide, superoxide- and peroxyradicals EPR spectra changes by influencing of plaferon LB.

The experimental data indicates that the basis EPR signal intensity of NO in Jurkat cells is  $12 \pm 1,0$  mm/mg, and the EPR signals of superoxide- and peroxyradicals are not revealed. After incubation with LPS for 18 ours in Jurkat cells culture the intensity of EPR signal of NO increases significantly and amount to  $32 \pm 2,0$  mm/mg, there are revealed EPR signals of  $O_2^-$  and LOO. In the case when Jurkat cells culture was incubated with LPS and plaferon LB, the intensity of EPR signal of spin-trapped NO is not changed in comparison to the basic meaning, intensity of EPR signal of  $O_2^-$  decreases significant and arranges 20% of LPS induced level, but LOO. EPR signal is not revealed EPR spectra of Jurkat cells culture.

It is generally accepted that freshly isolated human T cell produce NO with very low intensity (Pae H.-O., et al., 2004). LPS activates macrophages and induces IL-1, IL-6 and IFN-gamma production (Kiener P., et al., 1988, Raver J., et al., 1988). LPS also is a murine B lymphocyte mitogen and will activate protein kinase C and induce NF-kB in B cells although it has little effect on T lymphocytes (Sen R., Baltimore D., 1986). Many of these LPS-induced

effects are seen with nanogram or picogram per millilitre concentrations (Kornobluth R.S., Edgington T.S., 1986, Kiener P.F., et al., 1988). LPS has small ability for activate NF-kB in T cell for lack of an LPS receptor (TLR4) or of other reasons. (Pomerantz R., Jet al., 1990). However, Fise T. et al. showed, that TLR4 is present on the T cells and that LPS can increase the number of TLR4-positive cells in T cells culture (Fiset P.O., et al., 2003). As NF-kB is able to promote the expression of many pro-inflammatory genes (among them iNOSgene), Gertsch J., et al. studied influence of different mitogens on the transcription factor steady-state mRNA levels in different cells lines. It was shown, that PMA (phorbol 120 myrisate 13-acetate) treatment of Jurkat T- and PBM cells (peripheral blood mononuclear cells) time-dependent increased cytokine and transcription factor steady-state mRNA level. The use of LPS instead of PMA as stimuli showed that observed effects were comparable in quality to the results obtained after PMA stimulation in PBMCs, but LPS did not significantly induce pro-inflammatory gene expression in Jurkat T cells (Gertsch J., et al., 2003). The other authors (Jimenez J.L., et al., 2001) indicate, that HIV-infected T cells and Jurkat cells expressed iNOS mRNA.

As shows results of our experiments, with EPR method we revealed basic LPS inducible spin-trapped NO in Jurkat cells culture. Stimulation by LPS promotes generation of superoxide- and peroxyradicals. Decrease of this LPS-stimulated free radicals EPR signals intensity by influence of plaferon LB indicates, that this preparation affects on NF-kB' stability and by this way regulates proinflammatory genes expression and cells reactions on mitogens.

	NO	$O_2^-$	LOO
<b>Control</b>	$12 \pm 1,0$	-	-
<b>LPS</b>	$32 \pm 2,0$	$27 \pm$	$21 \pm$
<b>LPS+ PLB</b>	$11 \pm 1,5$	$7 \pm$	-

**Tab.1** study.

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## **Воздействие плаферона ЛБ на LPS-стимулированную генерацию свободных радикалов в культуре клеток Jurkat**

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### **Р Е З Ю М Е**

Оксид азота обладает различными физиологическими функциями: реагирует с различными клеточными компонентами, определяет переход клеток из физиологического в патологическое состояние и, таким образом, принимает участие в развитии различных патологических процессов. Поиск новых препаратов, способных селективно модулировать синтез NO, является актуальным. Исследовано воздействие препарата плаферон ЛБ на LPS-индуцированное образование оксида азота, свободных радикалов, кислорода и липидов в клеточной культуре клеток Jurkat. Методом ЭПР выявлен базисный сигнал ЭПР оксида азота в культуре клеток Jurkat. Установлено, что стимуляция клеточной культуры посредством LPS способствует усилению генерации оксида азота, свободных радикалов кислорода и липидов. Уменьшение интенсивности этих сигналов под действием Плаферона ЛБ указывает на то, что этот препарат влияет на стабильность NF- $\kappa$ B и этим путем регулирует экспрессию провоспалительных генов и реакцию клеток на митогены.

**Ключевые слова:** плаферон ЛБ, свободные радикалы, клетки Jurkat