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THE EFFEKT OF ERBISOL ON PRODUCTION OF IL-1β AND NITRIC OXIDE (NO) BY CELLS OF IMMUNE SYSTEM IN PATIENTS WITH CHRONIC UROGENITAL CHLAMYDIOSIS (CUGC) IN VITRO.

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ABSTRACT

Infection caused by Chlamydia trachomatis is on the special place among sexually transmitted diseases, due to high incidence of chronic urogenital chlamydiosis (CUGC), social importance of the problem and difficulties in treatment. In spite of an active use of new medicaments, the number of CUGC patients continues to grow therefore the search for new measures of immunocorrection is also in progress.

Aim: to determine in vitro the functional activity of cells of monocyte-and-macrophage series in CUGC patients by the production of IL-1β and nitric oxide under the action of immunocorrecting medicament Erbisol.

Materials and methods: the cytokine production was determined through studying the spontaneous and induced synthesis of IL-1β by cells of CUGC patients in vitro by means of immunoenzymatic method employing the test system DIACLONE (France) and immunoenzymatic analyzer STAT-Fax Plus-303 (USA). The content of stable metabolite NO-ionic nitrite-NO_2^- in supernatants of cells of monocyte and macrophage series was determined by means of spectrophotometry method using the standart Greiss-reagent.

Results: the IL-1β production by cells of healthy donors was 44.9±1.29 pg/ml, while the meanindex of spontaneous IL-1b production in CUGC patients was statistically higher than in healthies – 156.5±6.2 pg/ml. The activation of immunocompetent cells by mitogen in patients led to statistical reducing of monokin production and averaged 119.0±5.0 pg/ml ($p<0.05$). But the addition of Erbisol to the patient's immunocompetent cells stipulated the increase in IL-1b production almost 1,5 times, averaged 211.7±7.2 pg/ml and did not differ from the inducted cytokine production in healthy donors ($p> 0.05$). The spontaneous NO secretion in patients exceeded 2.5 times the NO level in healthy donors, the induction by Erbisol led to higher elevation of nitric oxide level in patients – about 3 times as compared with spontaneous production in healthies and 2 times exceeded the NO production induced by mitogen.

Conclusion: the lack of an increase in IL-1β production during activation by mitogens in CUGC patients in vitro, in contrast to healthies, can be considered as decrease in the reserve potential of cells of monocyte-macrophage series against the background of their high activation. Erbisol demonstrates the potential increase in IL-1β and NO production by patient's cells, that can be considered as the positive effect for the perspective empoying of the medicament in a situation when during a long time the immune system cannot ensure the effective resistance and destroy pathogen-Chlamydia trachomatis.

Key words: IL-1β, nitric oxide, chronic urogenital chlamydiosis

INTRODUCTION

The recent investigations in the immunology stipulated the development in the direction aimed at the problems of immunomodulation and based on the successful studying the mechanisms of functionary of the immune system and regulating the immune reactions. It particularly concerns the studying of functional activity of immunocompetent cells through analysis of production of cytokines which play a role of mediators fostering the cooperative intracellular interaction. Now the actual and perspective task is to develop medicaments with immunotropic specifity to be used in immunotherapy and immunorehabilitation. One of them is Erbisol and its analogues (Super Erbisol, Extra Erbisol and Erbisol). Erbisol is the representative of a new class of endogenic regenerative biological immunomodulatoring remedy elaborated in the scientific manufacturing centre "Erbis". Erbisol was received from the embrional tissue of cattle and comprises the complex of natural non-albuminous low-molecular organic compounds of non-hormonal origin.

One of the important factors of non-specific immunity is nitric oxide (NO) which is under active studying nowadays. It is produced by various cells: mononuclear phagocytes, neutrophils and also by hepatocytes, epithelial cells, gliacytes etc. It is known that the secretion of this factor into the intercellular space is going on not only in the presence of extracellular but also of intracellular infection with the aim to extract many pathogens (1).

We take an interest in a role of NO as an anti-inflammatory factor, as some authors demonstrated the ability of NO to penetrate into bacteria and fungi and to inhibit three groups of enzymes – ATP synthesis, Krebs cycle and DNA synthesis..

The scientists investigating the mechanisms of action of NO consider that when it interacts with superoxids then peroxide nitrite anion appears which plays an important role in cytotoxic effect of nitric oxide on microorganisms. Nitric oxide has specificity to affect both Chlamydia and other in tracellular microorganisms which are in epithelium. There is an interesting interrelation between NO and functional activity of lymphocytes. It was shown that nitric oxide stimulates the IL-2 production by T-helpers of type 1. In its turn, lymphocytes, cytotoxic in relation to Chlamydia trachomatis, are able to inhibit the grown of pathogen in the culture of epithelial cells, inducing NO-synthesis in them (4).

We grounded our studies on the concept that the changes available in the IL-1в production are a risk factor for further disorders in the link of immune reactions, which can lead to the development of chronic pathology (5).

The aim of the study was to experimentally define the mechanisms of the Erbisol action on the functional activity of immunocompetent cells by the production of IL-1β and NO in healthies and patients with chronic urogenital chlamydiosis (CUGC) for the elaboration of the scientifically grounded effective recommendations for its application.

MATERIALS AND METHODS

The separated on the standard gradient phicoll-verografin (1.076-1.078) mononuclear cells of peripheral blood were washed three times in the medium 199 and re-suspended in the culture medium RPMI-1640, which comprised 10% of embrional calf serum, 40 mg/ml of gentamicin, 5x10 M2 – mercaptoethanol and 3% L-glutamin. The cell suspension in concentration $1,5x10^6$ cell/ml was incubated 24h in CO_2 – incubator at 37° C 30 mg/ml, using various dilutions of Erbisol – 1:100, 1:500, 1:1000 and 10 000. The dilution 1:100 corresponded to the one-time dose of the medicament 2 ml in evaluation in the number of cells of lymphacytic-monocytic rank in 1 ml of the cultural medium (for $1,5x10^6$ cell/ml). When the incubation term was over, the cells were centrifuged at 1600 turns/min for 10 min, the supernatants were gathered together and stored for testing at - 20°C.

The IL-1β level in the supernatants was determined by means of immunoenzymatic method using the test system Diaclone (France). Testing was performed by the immunoenzymatic method ELISA on the STAT Fax 303 Plus device.

To determine the NO level, to 100 µl of the supernatant was added the Gress reagent in the some volume (1:1) as a color-forming agent to evaluate the reaction. The latter consisted of 1 part of 1% sulfanilamide, 1 part of 0.1% naftilethylenamide and the equal to them volume of 5% phosphoric acid. After the 10 min incubation, we determined the optical density of standards (titration of nitric oxide in cultural medium) and supernatant species on spectrophotometer at wave length 492-630 nm.

To statistically evaluate the results, the standard curve must be linear and indicate the directly proportional character of the ratio between the level of NO concentration in a supernatant and the units of optical density. The data of specios analysis in relation to the level of NO synthesis were determined by means of their interpolation with the received curve.

RESULTS

The investigations of the IL-1β production under the action of Erbisol were performed according to the above methods.

We set up the characteristics of the IL-1β producing ability of cells in patients with chronic urogenital chlamydiosis (44pts) in comparison with the data of 15 healthy donors. The IL-1β production was induced using both the mitogen LPS and the drug Erbisol.

The analysis of supernatants in healthies showed that the normal mean IL-1 production was 44.9±1.29 pg/ml. The activation of mononuclears by the LPS mitogen led to its increase up to 100.9±1.27 pg/ml (fig.1). Under the action healthy donors averaged 200.8±4.6 pg/ml, that is 4.5 times exceeded spontaneous and 2 times - IL-1β production under the effect of the mitogen LPS.

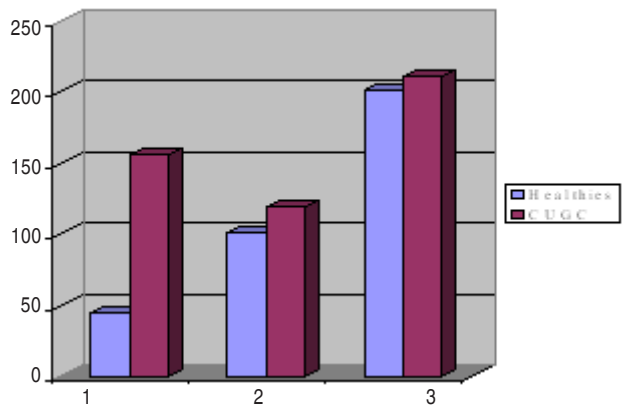

Fig. 1. IL-1β (pg/ml) production in healthies and CUGC patients – spontaneous (1) , mitogen-induced (2) and after the Erbisol effect (3).

The average spontaneous production of IL-1β in the CUGC patients was statistically higher than in healthies – 156.5±6.2 pg/ml. The activation of immunocompetent cells in patients led to statistical decrease in monokin production and averaged 119.0±5.0 pg/ml ($p<0.05$). But the addition of Erbisol to the immunocompetent cells of patients fostered

the increase in the IL-1b production almost 1.5 times, that averaged 211.7±7.2 pg/ml and did not differ from the induced production of cytokine in healthy donors ($p>0.05$) (Fig. 1).

So, the absence of the increase in the IL-1β production while in vitro activation by mitogens in the CUGC patients, in comparison with healthies, can be evaluated as a decrease in the reserved ability of cells of monocytic-macrophagal link against the background of their high activation. The drug Erbisol demonstrates the potentialities to elevate the IL-1β production by patient's cells that can be considered the positive effect in the situation when during a long time the immune system cannot provide the effective defence and destroy the pathogen – Chlamydia trachomatis.

Taking into consideration the above role of nitric oxide in pathogenesis of chlamydial infection, one of the tasks was to investigate this factor of oxygen independent mechanism of cell resistance to infection.

The production of nitric oxide was studied in 42 patients with CUGC and 15 healthy donors using the method based on the ability of polymorphonuclear leukocytes to synthesize NO from aminoacid L-arginin with the help of enzyme NO-syntetase which is produced by macrophages in response to bacterial endotoxins or anti-inflammatory cytokines (4).

In healthy donors the spontaneous NO secretion averaged 14.6±1.0 pg/ml. The mitogenic stimulation elevated the No secretion and was 17.6±1.2 pg/ml. The induction of cells by Erbisol in healthy donors led to enhancement of NO secretion, that exceeded the induction level by mitogen and was 23.0±1.0 pg/ml (Fig.2).

The data, presented on Fig. 2, testify to the high spontaneous and stimulated production of nitric oxide in patients with CUGC in comparison with the data in healthies. The spontaneous NO secretion in patients exceeded 2.5 times the NO level in healthy donors, and the stimulated one exceeded normal 3 times. The induction by Erbisol led to still higher elevation of the NO level in patients – about 3 times, when compared with spontaneous production in patients, and 2 times exceeded the mitogen-induced production of nitric oxide (Fig.2).

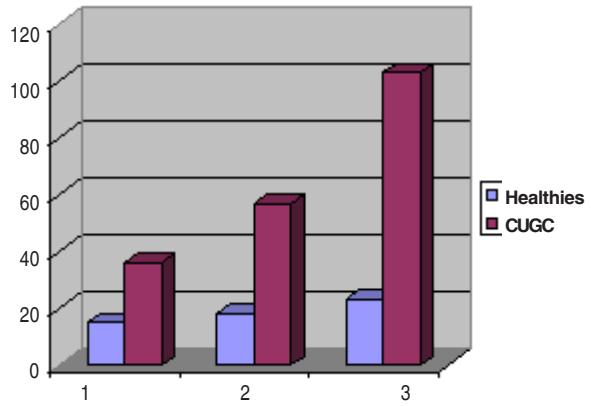

Fig. 2. NO production (pg/ml) in healthies and CUGC patients – spontaneous (1), mitogen-induced (2) and after the effect of Erbisol (3).

Our investigations showed that a new drug Erbisol is a stimulator for production of IL-1β and NO by cells in patients with CUGC, that is of great importance. Thus, Igietseme J. et al. (1997) showed that epithelial cells, after infecting with chlamydia, synthesize NO-sytetase which later promotes production of nitric oxide. The latter binds with superoxides, which are formed under the effect of bacterial endotoxins, damages a nucleus of bacterial cells and disturbs the functioning of mitochondria (3, 6).

Suggest that g-IF, which produces T-h, I type, induces the production in epithelium of NO-syntetase, and, respectively, nitric oxide which later influences chlamydia. The works of (6) confirmed that the interaction of epithelial cells and T-cells through adhesion of molecules promotes the NO production and chlamydia inhibition as a result of g-IF secretion by T-lymphocytes (2, 7).

It was revealed that in human epithelial cells r-IF is able to induce an enzyme indolamin-2,3-dioxigenase which catalyses the destruction of triptophan. It is considered that destruction of exogenetic triptophan leads to the inhibition of life cycle of chlamydia and the development of persistence (6).

Therefore, we consider the data, we have received about the effect of Erbisol, to be of great importance for elevation of counter chlamydial defense in patients with CUGC.

CONCLUSION

The resulting data about the high activity of oxygen dependent mechanisms of cells correlate with the above results which testify to the high secretion of IL-1в by cells of monocytic-macrophagal link. The application of Erbisol for stimulation of NO production shows that the compensatory reserve of cells is retained, in spite of the high production of nitric oxide in cases of urogenital chlamydiosis. We think that Erbisol demonstrated the immunomodulating abilities in the enhancement of the oxygen-independent mechanisms of killing of different pathogens that can be interesting to use it for successful elimination of a causative agent of chronic process.

REFERENCES

1. Николаенко А.Н. Международная заявка PCT/UA93/0003 "Биологически активное средство, способ его получения, препарат, содержавший указанное средство и способ использования препарата". 1993. [in Russian]

2. Битти В., Моррисон Р., Персистенция хламидий: от клеточных культур до патогенеза хламидийной инфекции. *Заболевания передающиеся половым путем.* 1995; 6:С. 3-24. [in Russian]

3. Малышев И.Ю. Введение в биохимию оксида азота. Роль оксида азота в регуляции основных систем организма. *Рос. журнал гастроэнтерологии, гепатологии, колопроктологии.* 1997; 1:С. 49-55. [in Russian]

4. Janeway CA, et al. Immunobiology: The Immune System in Health and Disease. New York, NY: Current Biology Publications; London, England: Elsevier Science London/ Garland Publishing, 1999.

5. Dinarello CA. Interleukin-1. *Rev Infect Dis.* 1984 Jan-Feb;6(1):51-95. [PubMed]

6. Blesson S, Thiery J, Gaudin C, Stancou R, Kolb JP, Moreau JL, et al. Analysis of the mechanisms of human cytotoxic T lymphocyte response inhibition by NO. *Int Immunol.* 2002 Oct;14(10):1169-78.Rev Infect Dis. 1984 Jan-Feb;6(1):51-95. [PubMed] [CrossRef]

7. Igietseme JU, Uriri IM, Chow M, Abe E, Rank RG. Inhibition of intracellular multiplication of human strains of Chlamydia trachomatis by nitric oxide. *Biochem Biophys Res Commun.* 1997 Mar 27;232(3):595-601. [PubMed] [CrossRef]

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