Indicators of cytokine regulation and pathogenetic ground for applying of Tivortin in patients with multidrug-resistant pulmonary tuberculosis

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Abstract. Production of IL-6 and IL-10 in TB patients is independent of drug resistance, but increases in response to increased synthesis of endotoxins by MBT; the magnitude of endogenous intoxication and cytotoxic hypoxia creates prerequisites for the development of drug resistant strains. The IL-18/IL-10 ratio in these patients characterizes the increase in severity of the patient's state, the spread of inflammation processes in the lungs and the development of drug resistance. Optimizing Tivortin pathogenetic therapy positively influences on the specific inflammation. It has been obsereved more rapid term of MBT cessation (an average of 3,8±0,7 months) in 75% of patients.

Keywords: *tuberculosis, multidrug-resistant, interleukins, Tivortin.*

Introduction. According to WHO Ukraine ranks 1-st in the ratio multidrug-resistant tuberculosis (MDR TB) among patients receiving re-treatment (79.4 %) [4]. In Ukraine MDR TB diagnosed in 16 % of patients who first diagnosed TB and 44 % of patients with recurrent disease.

Drug resistant TB – is the form in which the patient identifies Mycobacterium tuberculosis resistant to one or more anti-TB-drugs, as confirmed by a laboratory test drug susceptibility [3]. In patients with pulmonary TB drug resistance reaches 81 % [4]. Among the varieties the most concern is the stability MDR TB that may be the cause of extensively drug resistant TB (XDR).

It remains unclear the role of cytokines in the development of resistance of mycobacteria to anti-TB-drugs. Also, are not yet identified markers that reflect the progression of pathological process in multidrug-resistant tuberculosis, and was not set their predictive role in assessing the success of anti-tuberculosis chemotherapy in standardized programs [1, 2, 3].

Aim. Determine the features of cytokine regulation in patients with multidrug-resistant pulmonary tuberculosis and their role in development of the systemic inflammatory response, pathogenetic ground for applying of Tivortin.

Material and methods. Were enrolled 116 patients with pulmonary TB. All subjects were randomized in 3 study cohorts: cohort 1 (41 subjects) were included patients with newly diagnosed pulmonary TB, with preserved sensibility to TB drugs; cohort 2 (63 subjects) were included MDR TB patients with confirmed resistance to at least 3 first line TB drugs (HRS), cohort 3 (12 subjects) were included patients with XDR TB, control group (20 subjects) were included healthy humans Clinical, radiological, biochemical, microscopic, microbiological, immune-enzymatic and statistical study (ANOVA and Pearson correlation) methods were used.

The chemotherapy (ChT) program included 34 MDR TB patients, which are divided into two groups. Group 1 (gr.1 – control) included 18 patients treated with standard chemotherapy and hepatoprotector Karsil (1-2 tablets 3 times a day) for 2 months. Group 2 (gr.2 – main)) included 16 MDR TB patients who were administered nitrogen monoxide donator – Tivortin (producer LLC «Yria Farm»), 4.2% solution for infusion (100 ml intravenously daily per 10 days) then 10-day break with followed continuation for another 10 days. Subgroup A – before treatment; subgroup B – in treatment dynamics. Efficiency of optimized pathogenetic Tivortin therapy evaluated in dynamics for clinical symptoms, some blood biochemical parameters, cytokine balance, nitrogen monoxide level, chest X-ray data and the term of MBT cessation.

Results and discussion. We carried out a comparative analysis of certain pro- and anti-inflammatory cytokines (Chart 1) that shows a significant increase in the plasma concentration of cytokines in TB groups *vs.* control group, and we determined the probability of the dependence of these parameters upon the resistance profile of the MBT. So, the blood concentrations of IL-6 in all groups TB groups were significantly increased compared to control group, there was a 11.08 fold increase in group 1, 13.9 fold increase in group 2, and 4 fold increase in group 3 of IL-6 level ($p<0.001$). A significant intergroup difference was found of plasma concentration of IL-6 between patients with sensitive and resistant TB (Chart 1). Thus, the level of IL-6 in group 2 was 1.7 fold increased, compared to group1 ($p1<0.01$). However, in patients of group 3 marked reduction in IL-6 concentration was compared to group1 – 2.8 fold ($p<0.001$) and group 2 – 3.5 fold ($p_3<0.001$). Low values of IL-6 in patients XDR TB, in our opinion, can lead to chronic carrier of intracellular infection, rapidly progressive course of the inflammatory process, which poorly responds to anti-TB treatment and, probably, is one of the factors producing their own XDR TB forms due to prevalence of humoral immune responses [2, 3, 5].

A pronounced activation of all phases of the inflammatory niprocess in all study cohorts compared to control group, probably, is indicated by the increase in the level of anti-inflammatory IL-10 (Chart 1). Thus, in group 1 level of IL-10 increased by 2.3 folds, in group 2 – 1.8 folds, in group 3 – by 1.9 folds ($p<0.001$), this indicate on inhibition of cellular immunity and perhaps the beginning of specific chronic inflammatory process. The plasma concentration of IL-10 in sensitive TB patients has increased by 1.2 folds compared to group 2 and 3 ($p_1<0.001$, $p_2<0.01$). There were no statistically significant differences in concentration of IL-10 in group 2 and 3 ($p_3>0.4$).

Chart 1. Plasma concentrations of certain cytokines in sensitive and resistant pulmonary tuberculosis patients

Cytokines	Control group (n=20)	Group 1 (n=41)	Group 2 (n=63)	Group 3 (n=12)
IL-6* (pg/ml)	1.708±0.015	18.92±14.17 p<0.001	23.70±13.39 p<0.001 p_1<0.01	6.84±5.4 p<0.001 p_2<0.01 p_3<0.001
IL-10 (pg/ml)	1.79±0.127	4.2±0.75 p<0.05	3.38±0.79 p<0.001 p_1>0.001	3.55±0.23 p<0.001 p_2<0.01 p_3>0.4
IL-18 (pg/ml)	268.34±101.74	537.67±276.67 p<0.001	329.32±148.10 p<0.1 p_1<0.001	194.11±81.89 p<0.05 p_2<0.001 p_3>0.05

Note: Data are presented as average and standard error (M±m). p – significance level related to control group; p_1 – significance level between group 1 and 2; p_2 – significance level between group 1 and 3; p_3 – significance level between group 2 and 3. * – interleukine.

The activity of IL-18, whose role is to improve the resistance to intracellular pathogens and is essential for the formation of anti-TB acquired immunity, significantly increased in sensitive and MDR TB patients compared to control group. For example, in gropu1 there is a 2 fold IL-18 increase (p<0.001), respectively, in group 2 – 1.2 fold (p<0.1). However, in patients XDR TB there is a tendency to reduce the plasma concentration of this cytokine below the level of control group. The level of IL-18 in group 3 decreased by 1.4 folds in comparison with control group (p<0.05). Intergroup difference of plasma concentration of IL-18 in sensitive and MDR TB patients was proved. Thus, in group 2 *vs.* group 1 there is a 1.6 folds decrease of IL-18 (p_1<0.001), IL-18 decrease in group 3 *vs.* group 1 was of 2.7 folds (p_2<0.001). Also, there is a decrease of IL-18 concentration in group 3 of 1.7 folds compared to group 2 (p_3<0.05). The difference in plasma concentration of IL-18 in sensitive and MDR TB patients is on the ultimate level of statistical significance ($p_{1,2}$<0.001).

In our opinion, the production of IL-6 and IL-10 in TB patients, regardless of resistance, raise in response to increased synthesis of MBT endotoxins, increase of endogenous intoxication and cytotoxic hypoxia, all these create prerequisites for the development of resistance. What caused the lack of correlation between the IL is not yet clear, however, this fact is not crucial, as the most important prognostic criterion is the imbalance in the IL-18/IL-10 ratio; with an increase in the severity of the patient's condition, the spread of inflammation processes in the lungs and the development of drug resistance; there is a significant bulk of the Tx-lymphocyte type 2 (CD4+), which indicates the development of deep gap in cellmediated immune response and prevalence of an ineffective anti-inflammation immune activation. The increase in IL-6 plasma concentration, probably, indicates a high activity of systemic inflammatory response, which is maximally expressed in MDR TB patients (23.70±13.39). This cytokine plays a key role in the development of inflammation, immune response to infectious factor and lung tissue damage with the formation of massive destructive changes that were present in patients groups assessed by us. IL-6 plays a special role as „hepatocyte activating factor", which induce the synthesis of acute-phase proteins in the framework of systemic inflammatory response that leads to emerge of specific inflammation process outside the pulmonary tissue and activation of systemic inflammatory response syndrome.

High levels of IL-10 in patients with pulmonary TB have a favorable prognostic impact, because multifunctional properties of IL-10, ability to inhibit the synthesis of most proinflammatory cytokines and block apoptosis of macrophages and monocytes play an important role in the formation of a limited specific inflammation in the broncho-pulmonary parenchyma. Given the fact, those in MDR TB and XDR TB patients' levels of IL-10 is not too high and are efinitely lower than in newly diagnose TB patients, in such patients widespread, disseminated TB forms dominate over infiltrative forms (ratio 1:2).

The studies have shown that patients with MDR TB by Tivortin optimizing pathogenetic treatment better tolerability of ChT, more rapid detoxication (81.8% patients per 1,2±0,1 months) and bronchopulmonary (in 90.9% of subjects pert 2,7±0,3 months) syndromes were recorded.

Is established that inclusion Tivortin into the program of pathogenetic treatment while ChT intensive phase promotes the normalization liver functional activity, therefore low cytolytic activity of AST and ALT enzymes, prevents the development of drug-associated parenchymatous hepatitis, accompanied by the absence of clinical symptoms characteristic for acute toxicity liver disease (associated with the use of pyrazinamide and levofloxacin).

The results showed reduction of pro-inflammatory IL-6 and increase the ratio of anti- inflammatory IL-10 and IL-18 testify to the activation of the immune response Th-1 type, aimed, to restrict the specific inflammation within the affected site at Tivortin pathogenetic usage in the intensive phase of chemotherapy.

Refining pathogenetic therapy with the inclusion into the treatment program Tivortin enhances the effectiveness of standard chemotherapy, being characterized by the positive roentgenologic dynamics. So partial resorption of focal changes in the main group were in 62.5%; infiltrative – in 68.8%; destructive changes regression in – 75%; in the control group (gr.1B) was slight tendency to positive dynamics of X-ray changes within rentgencontrol period.

Microscopic and cultural studies in the control group observed a steady of MBT cessation only in 27.8% of cases, 72.2% – of MBT reverse by those who ChT inter-

rupted because of the development drug-induced liver injury in 83.3% of patients. Thus dyspeptic syndrome appeared in 13.3% of patients, asthenovegetative 20%, hepatomegaly in 26.7%, syndrome combinations were observed in 40%. In the main group subsequently detected a steady of MBT cessation in 100% of cases and, development of drug-induced liver injury was not observed.

Conclusions.

1. Assessment of IL-6 plasma concentration in pulmonary MDR TB *vs.* sensible TB patients revealed a significant 1.7 folds increase (p_1<0.01), and, respectively, a significant 1.2 folds decrease in the level of IL-10 and IL-18 (p_1<0.001), these confirm the strengthening of endogenous intoxication, cytotoxic hypoxia and activation «systemic inflammatory response» syndrome.

2. Assessment of plasma concentration of certain pro-andanti-inflammatory cytokines in MDR TB patients showed that it is dependent on the profile of MBT resistance to anti-TB drugs. Plasma concentration of IL-10 in MDR and XDR TB patients is significant lower than in sensible TB patients and correlates with the prevalence in MDR TB patients of widespread/disseminate TB forms over infiltrative TB forms (1:2 ratio).

3. It was established that the usage of Tivortin in patients with MDR TB prevents enhance of cytolytic activity of AST and ALT enzymes. Their level remained in the normal range (43.15±2.9 U/l and 37.97±2.3 U/l).

4. Comparative analysis of pro- (IL-6, IL-18) and anti-inflammatory (IL-10) cytokines in plasma contents of patients with MDR TB testify to the activation of the immune response Th-1 type, aimed, to restrict the specific inflammation within the affected lung at Tivortin pathogenetic usage in the intensive phase of chemotherapy.

5. Optimizing Tivortin pathogenetic therapy positively influences on the specific inflammation so partial resolution of focal changes occurred in 62.5% of cases; infiltrative – in 68.8%; destructive changes regression – in 75% of patients. It has been obsereved more rapid term of MBT cessation (an average of 3,8±0,7 months) in 75% of patients.

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Показатели цитокиновой регуляции и патогенетическое обоснование для применения Тивортина у больных с мультирезистентным туберкулезом легких

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Резюме. Продукция ИЛ-6 и ИЛ-10 в группах больных ТБ независимо от резистентности возрастает в ответ на повышение синтеза эндотоксинов МБТ, нарастание эндогенной интоксикации и цитотоксической гипоксии, создает предпосылки для развития их резистентности. Дисбаланс соотношения ИЛ-18/ИЛ-10 у этих больных характеризует нарастание тяжести состояния пациента, распространенность воспалительного процесса в легких и формирование резистентности.Оптимизация Тивортином патогенетической терапии положительно влияет на специфического воспаления. Прекращение бактериовыделения произошло в более быстрый строк (в среднем 3,8±0,7 месяцев) у 75% пациентов.

Ключевые слова: *туберкулез, мультирезистентный туберкулез легких, интерлейкины, Тивортин.*