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Application of the Specific Imunoglobulin Therapy in The HIV Infected Persons with Chronic Toxoplasmosis

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Abstract. **The objective** of the research was to study the effectiveness of complex treatment of HIV-infected patients with moderate chronical toxoplasmosis using azithromycin and human immunoglobulin against Toxoplasma Gondii. The patietns were not at the AIDS stage.

Materials and methods of the research. 60 patients with I, II and III clinical stage of the HIV-infection who had moderate chronic toxoplasmosis were under observation. Moderate form of toxoplasmosis was not considerated as AIDS indicator disease. The immunoenzyme method was used to determine titers of specific anti-Toxoplasma IgG and IgM, and IL-2, IL-4, IL-10, TNF-2α, IgM, IgA, IgG.

The I group of patients (30 individuals) received azithromycin inside for 21 days in a dose of 1.0 g/day, and the second group (30 persons) received azithromycin for 21 days in a dose of 1.0 g / day and human immunoglobulin against Toxoplasma Gondii every second day number 5 in a dose of 3.0 ml intramusculary.

Results. Substantial symptoms of the toxoplasmosis included swollen lymh nodes (90.0%), low-grade fever (63.33%), neurocirculatory dystonia (86.67%), moderate hepato-splenic syndrome (58.33%), weakness and fatigue (81.67%), muscle pain (36.67%), myocarditis (6.67%), chorioretinitis (3.33%).

Both groups of patients had positive clinical changes after treatment. The conditional score of toxoplasmosis activity (CSAT) was applied to illustrate the effectiveness of two treatment regimens more clearly. CSAT was the average percentage of six symptoms (fever, neurocirculatory dystonia, weakness, muscle pain, myocarditis, chorioretinitis). Before treatment CSAT constituted 46.39±6,4% and after treatment it decreased significantly to 16.67±6.8% only in patients who received azithromycin and human immunoglobulin against Toxoplasma Gondii ($P<0.05$).

Increased levels of IL-2, IL-4, IL-10, TNF-2α were detected in all the patients before treatment. After course of treatment with azithromycin and immunoglobulin the levels of IL-2 and TNF-2α decreased to normal in contrast to the patients who received only azithromycin.

Conclusions

1. Application of the specific human immunoglobulin against Toxoplasma Gondii increased the effectiveness of treatment of moderate chronic toxoplasmosis with the use of azithromycin in the HIV-positive patients not at AIDS stage. The effectiveness of treatment manifested in the decrease in toxoplasmosis symptoms complex.
2. The activity of proinflammatory Interleukin-2 and TNF-2α and anti-inflammatory Interleukins-4 and 10 increased in the HIV-infected persons with toxoplasmosis. After the treatment with azithromycin and human immunoglobulin against Toxoplasma Gondii the levels of IL-2 and TNF-2α decreased indicating the completion of immune inflammation process.

Keywords: HIV-infection, toxoplasmosis treatment, azithromycin, immunoglobulin against Toxoplasma Gondii.

Problem statement and analysis of the recent research

A primary contamination leads to generalization of toxoplasmosis infection during first 3-6 weeks. After the agent fixation the pseudocysts are formed in target organs under the pressure of host's immune system. Toxoplazma bradyzoites existing into pseudocysts are practically inaccessible for most anti-parasitic drugs and immunologlobulins. The antiprotozoic therapy of latent toxoplasmosis is unreasonable due to reduced metabolism of bradizoites and toxoplasma inaccessibility in the pseudocysts for drugs influence [4]. The c*hemotherapy* of acute or chronic

toxoplasmosis is reasonable because tahyzoites actively intracellularly reproduce and pass into the extracellular space. However, in this case chemotherapeutic agent also do not have good access to toxoplasma. Prolonged courses of the treatment with pirimetamin, sulfonamides, tetracyclines are accompanied by a toxic and potential teratogenic effects, so their use should be thoroughly thought over [4, 9]. Some antibacterial agents can create high concentration in the intracellular environment and might be important for the treatment of chronic toxoplasmosis. These drugs belong to the groups of macrolides and azalides – clarithromycin, spiramycin, azithromycin, clindamycin [5- 7, 12]. The HIV-infected persons with absent or moderate immunodeficiency may have the subclinical, mild or moderate severity of chronic toxoplasmosis like in the HIV-negative persons. The danger is that HIV-induced immunodeficiency will deepen over time and the barriers to intensive toxoplasma multiplication will then be eliminated. In this case the bradyzoites can be reactivated and transformated to tahyzoites with specific toxoplamosis destruction of the various tissues, namely chorioretinitis, lymphadenopathy, hepatitis, myocarditis and many other pathologies. Therefore, the severe form of toxoplasmosis, such as encephalitis, pneumonitis, septic with often fatal outcomes are observed in the patients with profound immunodeficiency (CD<100 in 1 ml of blood) [6]. Chemotherapy of severe toxoplasmosis in a patients with AIDS (cerebral toxoplasmosis) is defined by the Pprotocol of the Minisry of Health of Ukraine of 2007, which includes pirimetamin and sulfadiazine. However, only second-line agents such as clindamycin, azithromycin, clarithromycin are available in the clinic currently [7]. However, the monotherapy is less effective than combined with two drugs. The specific human immunoglobulin against Toxoplasma Gondii is also used for adults and children for improvement of treatment outcomes [2, 8, 9]. The immunoglobulins can neutralize pathogens and block their adhesion and penetration into the cells and promote elimination of the microorganism and thus often interrupt the infectious process. The most important function of antibodies is organization of cell-molecular mechanisms of immune system to implement of antibody-dependent cytotoxicity in a focus of Toxoplasma multiplication. Trophozoides opsonizated by antibodies and infested cells are recognized and destroyed by macrophages, natural killers, eosinophiles together with sensitized T-cells. In addition, the antibodies activate the complement system through the classical path that leads to destruction of microorganisms and modified human cells by osmotic lysis [2, 10]. The use of specific immunoglobulin against Toxoplasma Gondii in HIV-infected patients has not become widespread until now. Moreover, the protocol does not give recommendations for the treatment of mild and moderate forms of toxoplasmosis in patients with I-III stages of HIV-infection [7].

The objective of the research was to study the effectiveness of complex treatment of moderately severe form of chronic toxoplasmosis in the HIV-infected persons without the AIDS stage using azithromycin and anti-Toxoplasma Gondii human immunoglobulin.

Materials and methods of the research

60 HIV-infected persons (24 men and 36 women) at the age of 23-38 (mean age was 29 years) without AIDS stage (44 persons had I and II, and 16 had III clinical stage of HIV-infection) were under observation. Chronic toxoplasmosis of moderate severity was diagnosed.

18 patients received antiretroviral therapy (ART) for 1-5 years, 7 persons received substitution maintenance therapy of opiate addiction for 2-3 years. Pregnant women were not observed during the treatment. CD4 + T lymphocytes level was within 290-562 cells per 1 mcl of blood. The patients receiving ART had undetermined level of viral load (<20 RNA copies per 1 ml of blood) and those who did not receive ART had viral load within 32-69 thousand per 1 ml of blood. Diagnosis of toxoplasmosis was made using test kits “Diaprofmed” (Ukraine) for enzyme-linked immunosorbent assay (ELISA) determining titers of specific IgG and IgM. The active phase of Cytomegalovirus infection and Epstein-Barr-virus infections were excluded in the patients using the same methods and appropriate test systems. Both the pulmonary and extrapulmonary tuberculosis were excluded in patients as well. The interleukins (IL-2, IL-4, IL-10, TNF-2α) and immunoglobulins (IgM, IgA, IgG) were determined in the patients using the ELISA method and test kits “Vector-Best” (Russia). Digital material was processed using the Excel program with the use of Student’s t-test.

As the treatment of moderate form of toxoplasmosis is not provided by protocol for HIV-infected patients we proposed the scheme of toxoplasmosis treatment with azithromycin and specific immunoglobulin [7, 11]. The patients with clinical and laboratory sings of chronic toxoplasmosis were divided into two groups according to the treatment regime: I group (30 persons) received azithromycin in a dose of 1.0 g/day for 21 days; II group (30 persons) received azithromycin azithromycin in a dose of 1.0 g/day for 21 days and anti-Toxoplasma Gondii human immunoglobulin azithromycin in a dose of 3.0 ml intramuscularly every other day, №5. The clinical and laboratory monitoring of treatment efficacy was performed after completion of the antibiotic therapy.

Results of the research

Considering the significant difficulties of clinical and serological diagnosis of the active toxoplasmosis infection in HIV-infected persons, the diagnosis was made by determination of the possible symptoms of chronic toxoplasmosis and exclusion of the diseases with similar symptoms and syndroms [1, 3, 10]. As follows from the presented data in table 1, the significant symptoms of toxoplasmosis included low-grade fever (63.33%), neurocirculatory dystonia such as headache, hypotension, discomfort in the heart area, tachycardia, irritability (86.67%), weakness, and fatigue (81.67%), moderate hepato-splenic syndrome (58.33%), muscle pain (36.67%), the electrocardiographic signs of cardiosclerosis (31.64%), myocarditis (6.67%), chorioretinitis (3.33%), which was combined with old scarring changes of the fundus of eye after previes inflammation without case history or the optic nerve degeneration (1.67%). The titer of anti-toxoplasma IgG higher than 200 IU/ml was considered significant for the diagnosis of the active toxoplasmosis [3], which was found in all patients. The combination of 3 aforementioned symptoms and the antibodies against Toxoplasma IgG present in titer >200 U/ml were the basis for diagnosis of the moderate severity form of chronic toxoplasmosis [10].

Table 1

Manifestations of the moderate form of chronic toxoplasmosis in the HIV-infected persons

Symptoms	Before treatment		After treatment Group I		After treatment Group II	
	N=60		N=30		N=30	
	Abs	%	Abs	%	Abs	%
Swollen limph nodes	54	90.0±3.9	26	86.67±6.2	25	83.33±6.8
Low-grade fever	38	63.33±6.2	6	20.0±7.3 P<0.001[4]	2	6.67±4.6 P<0.001
Neurocirculatory dystonia (headaches, fluctuating blood pressure, heart pain, tachycardia, irritability)	52	86.67±4.4	14	46.67±9.1 P<0.001	6	20.0±7.3 P<0.001 P_1 <0.05[5]
Mild hepato-splenic syndrome Weakness	35	58.33±6.4	16	53.33±9.1	19	63.33±8.8
Muscle pain	49	81.67±5.0	17	56.67±9.0	6	20.0±7.3
Cardiosclerosis				P<0.05		P_1<0.01
Myocarditis	22	36.67±6.2	4	13.33±6.2	1	3.33±3.3
Chorioretinitis	19	31.67±6.0	12	40.0±8.9	11	36.67±8.8
Residual symptoms after past	4	6.67±3.2	0	0	0	0
chorioretinitis	2	3.33±2.3	1	3.33±3.3	0	0
Optic nerve degeneration	2	3.33±2.3	1	3.33±3.3	2	6.67±4.6
	1	1.67±0.7	1	3.33±3.3	0	0
CPAT, %	46.39±6.4		23.33±7.7 p>0.05		16.67±6.8 p<0.05	

Note. Group I – group of patients receiving only azithromycin;

Group II – group of patients treated with azithromycin and immunoglobulin against Toxoplasma gondii;

CPAT – conditional point of active toxoplasmosis;

p – statistical significance of difference between the values of this index before and after treatment;

p_1 – statistical significance of difference between the values of this index in the both groups of patients after treatment.

Positive clinical changes were observed in both groups of the patients after the treatment. The main sign of recovery was low-grade fever disappearance, however it remained only in some individuals (in 20.0% of patients in group I and in 6.67% of patients in group II), which was associated with not only toxoplasmosis infection but other opportunists or conditions which were not found at that time.

The neurocirculatory dystonia symptoms decreased but they kept in some patients, namely in 46.67% of patients in group I and in 20.0% of patient in group II (p_1<0.05). After treatment the complaints of weakness remained in 56.67%

of the patients in group I and 20.0% in the group II (p_1<0.05), muscles pain remained in 13.33% of patients in group I and in 3.33 % of patients in group II. The symptoms of myocarditis disappeared in all patients after the treatment, however myocardiosclerosis phenomena remained. Thus, the amount of persons with myocardiosclerosis increased to 40.0% in the group I and to 36.67% in the group II (Table 1). The hepato-splenic syndrome remained in all patients in both groups after treatment, but the liver size slightly decreased. The polilymphadenopathy symptoms remained practically unchangeable as they were associated with not only toxoplasmosis but with HIV-infection primarily. At the end of treatment with azithromycin and immunoglobulin the visual signs of inflammation on the fundus disappeared completely in the patient with chorioretinitis, however the changes in the fundus remained in other patient with similar disorders who received only azithromycin what made us continue treatment up to 6 weeks. Determination of effectiveness of the toxoplasmosis treatment based on certain nonspecific symptoms was unconvincing. Therefore, the conditional point of active toxoplasmosis (CPAT) was used in each group of the patients. CPAT was calculated as average percentage of the six topical symptoms or syndroms: low-grade fever, neurocirculatory dystonia, weakness, muscle pain, myocarditis and chorioretinitis (Table 1). So, the rate of CPAT in the whole group patients was 46.39±6.4% before treatment and it decreased to 23.33±7.7% after treatment (p>0.05) in the patients of group I and to 16.67±6.8% (p<0.05) in the patients of group II who received the specific immunoglobulin additionally. CPAT reduction after treatment reflected the effectiveness of treatment both in group I and II of the patients, but only in those who were treated with azithromycin and specific immunoglobulin; CPAT reduction was significant comparatively with this data before treatment.

The changes of immunological parameters such as interleukins 2, 4, 10 and TNF and three classes of immunoglobulins (IgG, IgM, IgA) were studied before and after treatment. According to the data presented in table 2, the elevated levels of IL-2
(4.79±0.33 pg/ml, compared to 2.70±0.37 pg/ml in the donor, p_1 <0.001) and TNF-2α (4.22±0.29 pg/ml, compared to 1.9±0.44 pg/ml in the donor, p <0.001) were found in all patients compared with healthy people. Elevated levels of anti-inflammatory cytokines IL-10 (9.86±1.14 vs 6.70±0.73 in the donors, p <0.01) and IL-4 (2.38± 0.34, compared to 0.81±0.09 in the donors, P <0.001) were also observed. After a course of the treatment with azithromycin and immunoglobulin the levels of IL-2 decreased to normal level (2.37±0.26 pg/ml, compared to 4.79±0.33 pg/ml before treatment, P_1<0.05, while indicator in healthy individuals constituted 2.70 ± 0.37 pg/ml, p>0.05) and TNF-2α decreased to normal level (2.20±0.14 pg/ml, compared to 4.22±0.29 pg/ml before treatment, p_1<0.001, while the indicator in healthy individuals constituted 1.90±0.44 pg/ml, p>0.05). The levels of IL-10 and IL-4 did not undergo significant changes after treatment. In the group of persons who received only azithromycin at repeated examination at the end of treatment the levels of all studied interleukins did not undergo significant changes and remained higher than normal. Comparing the parameters of both groups of the patients after treatment, we observed the lower levels of proinflammatory cytokine IL-2 (2.37±0.36 vs 4.15±0.56, p_2<0.05) and TNF-2α (2.20±0.14 pg/ml vs 3.46±0.39 pg/ml, p_2<0.001) in the patients who received immunoglobulin additionally than in other group who received azitromicin only. Such dynamics of proinflammatory interleukine indicated the completion of immunological reactions and inflammation in a locus of active toxoplasmosis process in group of the patients who were treated with azithromycin and immunoglobulin.

During treatment the levels of major classes of immunoglobulins (IgM, IgG, IgA) had no significant changes (Table 2).

Table 2

The Immunological parameters in the HIV-infected patients before and after treatment of the chronic toxoplasmosis

Parameters	Groups of the HIV-infected patients			
	Donors n=30	All patients before treatment n=60	After treatment Group I n=30	After treatment Group II n= 30
IL-2 (pg/ml)	2.70±0.37	4.79±0.33 p<0.001	4.15±0.56	2.37±0.36 p_1 <0.001 p_2 <0.05
IL-4 (pg/ml)	0.81±0.09	2.38±0.34 p<0.001	1.64±0.90	1.59±0.77
IL-10 (pg/ml)	6.70±0.73	9.86±1.14 p<0.01	6.98±1.81	8.18±2.03
TNF-2α (pg/ml)	1.90±0.44	4.22±0.29 p <0.001	3.46±0.39	2.20±0.14 p_1<0.001 p_2<0.001

IgG (g/l)	10.3±3.14	15.72±2.38	16.82±3.81	17.90±3.01
IgA (g/l)	2.51±1.12	2.22±0.50	2.04±0.31	3.17±0.53
IgM (g/l)	2.31±0.37	2.09±0.65	2.05±0.28	2.76±0.27

Note. p – statistical significance of difference between the values of this index in group I or II and donors; p_1 – statistical significance of difference between the values of this index in group I or II after and before treatment; p_2 - statistical significance of difference between the values of this index in groups I and II after treatment.

Discussion of the results

Chronic toxoplasmosis is often reactivated in HIV-infected persons, but its severity depends from the degree of immunodeficiency. Patients who do not have deep immunosuppression undergo the course of the disease similar to that in the HIV-negative patients. Adequate treatment which was conducted for these patients had great importance for prevention of severe brain damage – toxoplasmosis encephalitis. Antibiotics with action against Toxoplasma can be effective, but the disease relapse prevention may fail on the background of deepening immunodeficiency. The human immunoglobulin against Toxoplasma Gondii was included to the treatment regimens in order to improve the quality of recovery. Reduction of symptoms and regulation of the cytokine status of the patients' immune system was observed as a result of its use. The increased levels of proinflammatory cytokines IL-2 and TNF-2α observed in patients suggested the immune inflammation process in response to active reproduction of Toxoplasma in the body tissues. The elevated levels of anti-inflammatory cytokines IL-4, IL-10 observed in HIV-infected patients created a favorable ground generally for opportunist microorganisms growth [6]. Reduction of proinflammatory cytokines activity in patients receiving immunoglobulin can be considered as an important criterion of the complete recovery because they reflected the attenuation of specific inflammation. However, the levels of anti-inflammatory cytokines IL-4 and IL-10 were continuously enhanced in both groups of the patients. These cytokines had strong inhibitory effect on specific cell-cytotoxicity in relation to infected cells with viruses and parasites and created the favorable conditions for further progression and recurrence of opportunistic infections including the chronic toxoplasmosis.

Conclusions

1. Application of the specific human immunoglobulin against Toxoplasma Gondii increased the effectiveness of moderate chronic toxoplasmosis treatment with azithromycin in the HIV-positive patients who did not have AIDS stage. The effectiveness of treatment manifested in the decrease in toxoplasmosis complex symptoms.
2. The activity of proinflammatory Interleukin-2 and TNF-2α and anti-inflammatory Interleukins-4 and 10 increased in the HIV-infected persons with toxoplasmosis. After the treatment with azithromycin and anti-Toxoplasma Gondii immunoglobulin the levels of IL-2 and TNF-2α decreased indicating the completion of immune inflammation process.

Prospects for further research

The immunoglobulins against Toxoplasma used passively are an effective means of treatment in a short period only, as the protective antibodies disappear after brief circulation. Due to the complex immunopathology (the reduction of CD4 + T lymphocytes and the increasing levels of proinflammatory and anti-inflammatory cytokines) the favorable ground for the toxoplasmosis reactivation is created. Therefore, further research should be conducted to search for the new medicine with more beneficial impact on cytokine balance of immune system.

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