Effect of Combination of Anticytokine Preparations Anaferon and Artrofoon on Immune Inflammation in Rheumatoid Arthritis

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Treatment with a combination of artrofoon and anaferon significantly reduced clinical and laboratory parameters of rheumatoid inflammation and significantly decreased the content of antiinflammatory cytokines in the blood. These findings attest to antiinflammatory and immunomodulating effects of these preparations.

Key Words: *rheumatoid arthritis; cytokines; anticytokine therapy; artrofoon; anaferon*

Current approaches to pharmacotherapy of rheumatoid arthritis (RA) are based on modern concept on the leading mechanisms of its development. Pathogenetic peculiarities of rheumatoid inflammation provided the basis for the creation and clinical application of a new class of antirheumatic drugs, belonging to biological agents. Infliximab (remikeid), the first preparation recommended for the therapy of RA, contains chimeric murine monoclonal antibodies to TNF-α.

However, despite introduction of biological agents into clinical practice, at least 30% patients do not respond to the treatment because their low efficiency, intolerability, low treatment compliance. Generally, the side effects in 42% patients result from discontinuation of treatment with disease-modifying preparations [5]. As for anti-TNF-α therapy, serious side effects develop in each fifth patient receiving infliximab or etanercept [8-11]. Anticytokine therapy has a number of contraindications. Moreover, foreign anticytokine drugs are expensive.

These problems limit the use of these preparations in clinical practice in Russia. In this context, the development of new drugs on the basis of antibodies to TNF-α characterized by high efficiency and good tolerability is an urgent problem.

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Russian scientists created a new class of preparations on the basis of ultralow doses of antibodies to endogenous regulators [4]. Among them, artrofoon and anaferon are promising preparations for the treatment of RA.

The efficiency of artrofoon in the treatment of joint disease was studied in various research centers in Russia [1-3]. It was found that course treatment with artrofoon significantly reduces clinical manifestations of RA, urogenic arthritis, spondyloarythritis, and osteoarthroosis. Moreover, long-term therapy with artrofoon decrased blood level of proinflammatory cytokines in patients.

Pharmacodynamics of anaferon underlying its immunomudulating effect consists in induction of Th1- and Th2- immune responses. Anaferon stimulates production of Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-10) cytokines, normalizes the Th1/Th2 balance, stimulates production of IFN-α and IFN-β by lymphocytes, and normalizes the content of different subpopulations of lymphocytes in the peripheral blood [4]. Anaferon exhibits pronounced antiviral activity due to activation of natural mechanisms of antiviral immunity. This phenomenon is of particular importance in light of the discussed role of viral infection in the initiation of the immunopathological process in RA.

These data pathogenetically substantiate combined application of these two preparations modifying the

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content of TNF-α (artrofoon) and IFN-γ (anaferon), the key mediators of the immune inflammation.

MATERIALS AND METHODS

We performed a double-blind randomized placebo-controlled study including 60 patients (51 women, 85%, and 9 men, 15%) aging 24-75 years, (mean age 58.59±1.34 years) with documented RA. The patients were divided into 2 groups depending on the therapy. Group 1 patients received combined therapy with artrofoon and anaferon in a daily dose of 8 tablets each drug; group 2 patients received placebo (sublingually).

The efficiency of treatment was evaluated after 1, 3, and 6 months by the dynamics of the major parameters included into international recommendations for clinical studies of disease-modifying preparations for the treatment of RA: time of morning stiffness, tender joint count (of 68 and of 28 joints), swelled joint count (of 66 and of 28 joints), integral assessment of joint pain, general health status evaluation, assessment of disease activity by the physician, assessment of functional activity of the patient by Health Assessment Questionnaire (HAQ), total disease activity index (DAS-28), ESR, and contents of C-reactive protein (CRP) and rheumatoid factor (RF). The content of proinflammatory cytokines was measured by immune-enzyme assay (Proteinovyi Kontur).

Comparison of the initial severity of clinical symptoms and laboratory shifts revealed no significant differences between the groups, which confirmed the identity of the studied groups by clinical manifestations and severity of RA. Background therapy with disease-modifying preparations and nonsteroidal anti-inflammatory drugs was also comparable.

RESULTS

No significant changes were noted for all studied parameters in both groups 1 month after the start of therapy. In group 1, no negative dynamics of clinical and laboratory parameters were noted, which attests to stabilization of the immunoinflammatory process even after 1-month course of anticytokine therapy. In group 2 patients, the majority of the test parameters did not improve and even tended to worsen.

After 3 months of combined therapy with artrofoon and anaferon, the time of morning stiffness considerably decreased (by 30 min on average), though the difference from the initial value was insignificant. A significant positive dynamics was observed for all parameters except general health status evaluation and functional activity by HAQ. Improvement of clinical symptoms was associated with positive shifts in the major laboratory parameters. We observed a significant decrease in ESR and CRP level, the content of RF also tended to decrease. The content of all proinflammatory cytokines significantly decreased (Table 1).

In group 2, no significant positive shifts in clinical and laboratory parameters were noted by the 3rd month of therapy. The tendency to worsening of some parameters was noted, *e.g*. we observed an increase in tender joint count (by 2 of 66 joints and by 2 of 28 joints on average) and swelled joint count (by 1 of 66 joints and by 1.7 of 28 joints). Evaluation of disease activity by VAS and by DAS-28 index also revealed aggravation of this parameter (by 7.5 mm and 0.5 points, respectively). ESR and the levels of CRP and RA also tended to increase. Blood concentrations of TNF-α, IL-1, and IL-6 in patients also increased. These negative changes in the studied parameters in this group did not reach the level of significance, but the absence of positive dynamics (even insignificant) distinguished this group from group 1 patients (Table 1). After 3 months, significant difference between the groups was noted for the following parameters: tender joint score (of 68 joints), general health status assessment by VAS, DAS-28, ESR, levels of CRP, TNF-α, IL-1, and IL-6. All these parameters in group 1 were better that in group 2.

After 6-month treatment course, we observed significant improvement of all parameters in group 1, except general health status (HAQ) and RF content; the dynamics of these two parameters was insignificant. It should be noted that the positive dynamics of the cytokine profile attained after 3-month treatment was maintained also after 6-month course. The level of all three proinflammatory cytokines was significantly below the initial values, the decrease in IL-1 content was most pronounced (Table 1) In group 2, no significant shifts in the studied clinical and laboratory indexes including the parameters of the cytokine profile were noted.

Comparison of final parameters in both groups showed that the results of treatment in group 1 were better by all studied parameters except HAQ score and RF content.

Evaluation of drug tolerability showed that side effects were noted in 15% patents of group 1 and in 10% patients of group 2. These were dyspepsia symptoms, headache, and urticaria. These side effects did not require discontinuation of the treatment, because they completely disappeared after reducing the dose. The exception was 2 patients with urticaria, in whom allergic reaction disappeared only after drug withdrawal; these patients were excluded from the study.

Thus, the study of pharmacodynamics of artrofoon and anaferon in their combined application in patients with RA showed antiinflammatory and immunomodulating effects of these drugs. The effects of the studied

TABLE 1. Clinical and Laboratory Parameters in Patients with RA after 3- and 6-Month Treatment Courses (*M*±*m*)

Parameter	Group 1			Group 2		
	initial	after 3 months	after 6 months	initial	after 3 months	after 6 months
Morning stiffness, min	131.80±12.81	100.00±13.32	93.60±11.88*	128.00±16.52	129.50±13.39	131.10±15.43
Tender joint count of 68	27.20±2.41	19.60±2.06*	19.30±1.96*	27.50±4.26	29.30±3.86+	31.00±3.96+
Swelled joint count of 66	15.00±1.57	10.40±1.61*	8.90±1.16*	13.40±2.51	14.50±2.31	15.60±2.65+
Tender joint count of 28	15.10±1.19	11.50±1.25*	10.7±11.00*	13.10±1.62	15.8±1.8	16.50±1.68+
Swelled joint count of 28	8.70±0.89	6.00±0.93*	5.60±0.71*	7.50±1.38	9.30±1.47	9.50±1.46+
Patient's assessment of pain syndrome by VAS, mm	60.50±3.48	48.90±4.16*	40.5±35.0*	58.10±4.19	58.5± .48	62.20±3.42+
Patient's assessment of general health status by VAS, mm	57.40±3.02	47.40±3.84*	48.00±2.94*	58.20±4.26	60.10±3.87+	59.00±4.02+
Physician's assessment of disease activity by VAS, mm	54.90±2.77	46.00± .04*	46.30±2.53*	48.0±4.6	55.60±4.47	57.9±4.59+
Health status and functional activity of the patient, HAQ score	49.50±2.13	48.30±2.14	46.40±1.95	52.20±3.68	52.00±3.68	51.80±3.66
Disease activity DAS-28	6.10±0.18	5.30±0.22*	5.30±0.21*	6.0±0.3	6.50±0.28+	6.60±0.28+
ESR, mm/h	48.30±1.95	42.30±1.92*	41.20±1.77*	46.00±2.99	50.50±2.68+	51.20±2.61+
CRP, mg/liter	15.50±1.79	10.70±1.47*	10.30±1.26*	15.20±2.49	19.60±3.27+	20.20±3.32+
RF, U/ml	27.10±3.23	23.20±3.36	20.41±2.66	24.00±4.74	26.10±4.93	26.53±4.86
TNF-α, pg/ml	535.60±41.19	385.80±32.04*	327.3±24.3*	542.60±33.73	618.50±34.59+	608.40±29.73+
IL-1, pg/ml	2297.0±104.8	1658.0±105.4*	1404.00±87.72*	2564.0±145.8	2558±159+	2534.0±128.6+
IL-6, pg/ml	561.7±20.1	456.8±16.6*	392.50±13.73*	597.70±32.96	635.00±29.69+	616.30±31.77+

Note. $p<0.05$ compared to: *initial values, +group 1.

preparations of antibodies to TNF-α and IFN-γ on cell mechanisms of immune inflammation were confirmed by the dynamics of the key inflammatory mediators TNF-α, IL-1, and IL-6. The mechanisms underlying the effects of antibodies in ultralow doses are most likely related to modification of cytokine structure or to a decrease in sensitivity of cytokine receptor, rather than to blockade of the corresponding cytokines. It can not also be excluded that ultralow doses of exogenous antibodies can stimulate the production of natural antibodies to proinflammatory cytokines.

The results of our clinical study suggest that the decrease in the content of TNF-α in patients with RA treated with a combination of artrofoon and anaferon can result not only from the direct effect of antibodies to TNF-α on activity of this cytokine, but also from reduced activity of endogenous IFN-γ regulating production of TNF-α by macrophages and monocytes. The advantage of this combination is potentiation of the therapeutic effect and the possibility of attained clinical improvement at earlier terms.

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