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Experimental and Clinical Study of the Effect of Artrofoon on Proinflammatory Cytokine Production

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The effect of Artrofoon on the production of proinflammatory cytokines was evaluated in experiments on mice with collagen-induced arthritis and in a clinical study on patients with rheumatoid arthritis. Artrofoon produced an antiinflammatory effect on animals with collagen-induced arthritis and reduced clinical signs of inflammation in patients with rheumatoid arthritis. These changes were accompanied by a significant decrease in the production of tumor necrosis factor-α and interleukin-1β.

Key Words: *Artrofoon; rheumatoid arthritis; ultralow doses; tumor necrosis factor-α; proinflammatory cytokines*

Rheumatoid inflammatory diseases, including rheumatoid arthritis (RA), are related to dysregulation of the immune system [4]. The Th1 immune response predominates in RA and is characterized by overproduction of proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). The antiinflammatory and analgetic effects of several drugs, including glucocorticosteroids (GCS) and monoclonal antibodies against TNF-α (Infliximab), are mainly associated with the modulatory influence on the production of proinflammatory and antiinflammatory cytokines (*i.e.*, inhibition of TNF-α and IL-1) [1].

The preparation Artrofoon (Research-and-Production Company "Materia Medica Holding") contains ultralow doses of antibodies against TNF-α. Previous experimental and clinical studies showed that this preparation exhibits high antiinflammatory activity [2,3]. Here we performed an experimental and clinical study of the effect of Artrofoon on proinflammatory cytokine production.

MATERIALS AND METHODS

Experiments were performed on 200 CBA/CaLac mice weighing 18-20 g. Collagen-induced arthritis (CIA) was caused by subplantar injection of type II collagen (single dose 100 μg, Sigma) in 50 μl complete Freund's adjuvant (ICN) into the right hindlimb [5]. Experimental animals were divided into 3 groups (64 mice per group). The animals of treatment group 1 intragastrically received Artrofoon (0.2 ml) for 14 days. Prednisolone (Nikomed) in a daily dose of 53 mg/kg was administered to mice of the treatment group 2 for 11 days. Control animals intragastrically received distilled water (0.2 ml) for 14 days. All preparations were administered 1 day before CIA. The reference group included 8 intact mice.

Eight mice of each group were killed 3 h after collagen injection (days 1, 3, 5, 9, 13, 17, and 21).

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We studied the inflammatory response and measured the concentration of proinflammatory cytokines in blood plasma and supernatants of peritoneal macrophages. The inflammatory response index (IRI) was calculated as follows:

$$IRI\ (\%) = (P_T\text{-}P_C)/P_C \times 100\%,$$

where P_T and P_C are weights of treated and control limbs, respectively. The concentration of proinflammatory cytokines TNF-α and IL-1β was measured by enzyme immunoassay (EIA, Amersham Pharmacia Biotech).

An open randomized comparative clinical study for the effectiveness and safety of Artrofoon in RA was performed with 80 patients of 18-70 years (72 women and 8 men; average age 52.4 years, 23-62 years; average duration of disease 10 years, 6 months-25 years). These patients had the articular form of RA (grades I-II, roentgenologic stages I-III) without systemic manifestations of the disease. RA of grade II was found in 80% patients. Roentgenologic stage II was found in 50% patients. Treatment with nonsteroid antiinflammatory drugs and basic therapy were performed in 85 and 14% patients, respectively.

All antiinflammatory drugs and/or local therapy with GCS were withdrawn 48 h or 1 month before the start of study, respectively. Artrofoon (n=50, 2 tablets) was given 4 times a day for 6 months. Patients of the reference group perorally received Diclophenac (n=30) in a daily dose of 100 mg for 6 months. The effectiveness of therapy was evaluated after 3 and 6 months. The concentration of TNF-α and IL-1β in blood plasma was measured by EIA (Proteinovyi kontur).

We calculated the mean value and standard error. The results were analyzed by parametric (Student's t test) and nonparametric tests (Mann—Whitney test and Wilcoxon test).

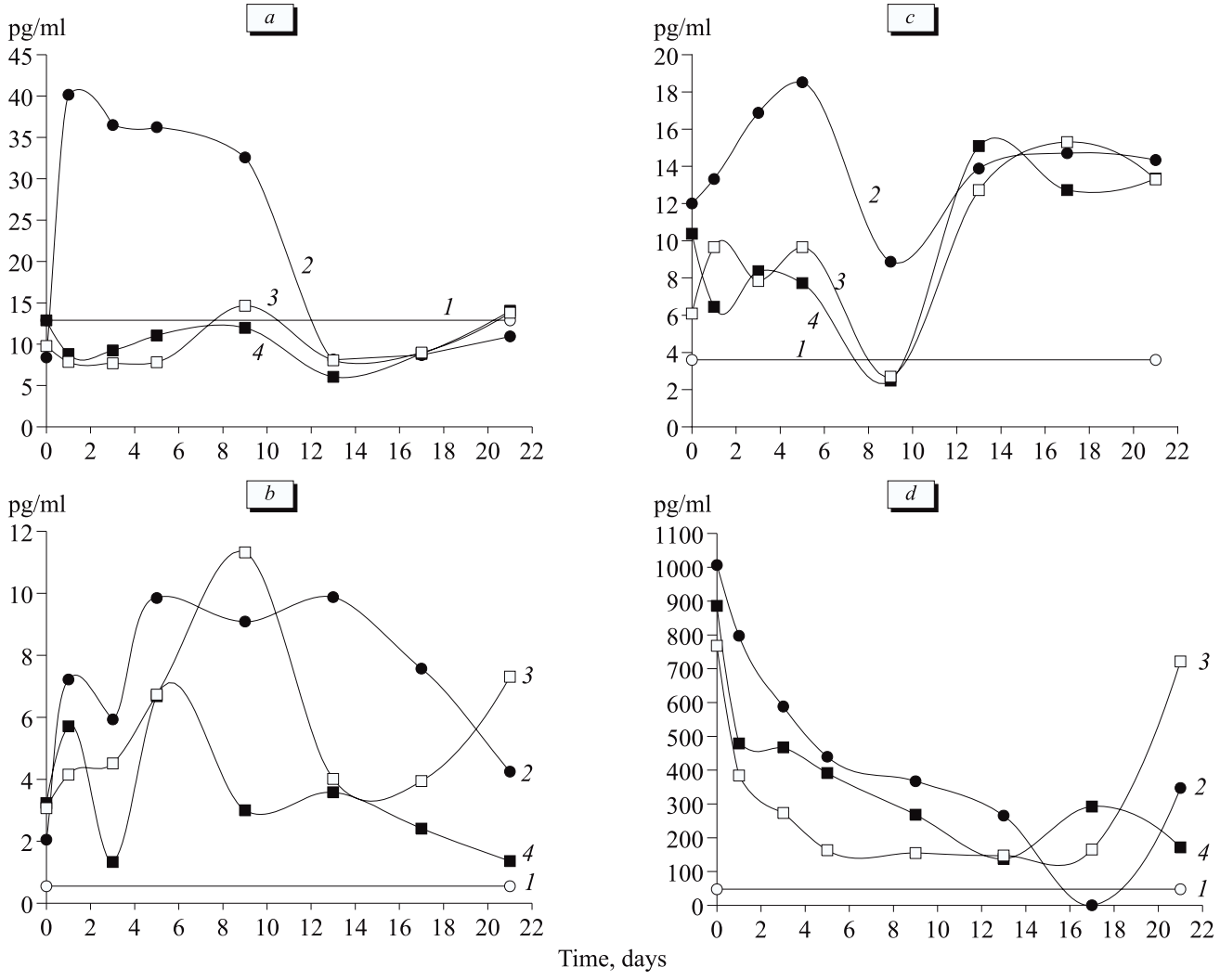

Fig. 1. Dynamics of proinflammatory cytokines in blood plasma (*a*, *b*) and supernatants of peritoneal macrophages (*c*, *d*) in CBA/CaLac mice with collagen-induced arthritis. Concentrations of TNF-α (*a*, *c*) and IL-1β (*b*, *d*).

RESULTS

Subplantar injection of type II collagen in complete Freund's adjuvant was followed by the development of RA in mice. IRI in control animals underwent phasic changes. The degree of right limb edema in control mice was highest on days 1, 5, 9, and 21. Artrofoon decreased IRI on days 1-13. However, statistically significant decrease in IRI was observed only on day 5. IRI in prednisolone-receiving animals significantly decreased on days 1-9 (compared to control mice and specimens of the Artrofoon group, Table 1).

Figure 1 illustrates the dynamics of proinflammatory cytokines in blood plasma and supernatants of peritoneal macrophages from CBA/CaLac mice.

The time of attaining the maximum concentration (C_{max}) of TNF-α in blood plasma of control mice was 24 h. The maximum concentration of TNF-α in blood plasma of control mice was 3.12-fold higher than in intact animals ($p<0.05$). The concentration of TNF-α in blood plasma of control mice decreased slowly. The concentration of TNF-α in blood plasma of control mice was 2.81 times higher than in intact animals on day 9 ($p<0.05$), but decreased to the baseline level on day 13. Prednisolone promoted the decrease in plasma TNF-α concentration in blood plasma on days 1-9 ($p<0.05$ compared to the control). The concentration of TNF-α in Artrofoon-treated mice was much lower than in control animals on days 1-13 ($p<0.05$).

Administration of Artrofoon and prednisolone to experimental animals was followed by a decrease in TNF-a production by peritoneal macrophages ($p<0.05$ compared to the control).

TABLE 1. Effect of Artrofoon on IRI in Mice with Collagen-Induced Arthritis (%, *M*±*m*)

Period of study	Experimental groups		
	Control	Artrofoon	Prednisolone
3 h	28.26±2.78	21.67±1.94^{+}	16.06±1.53*
Days			
1	35.05±1.27	30.61±1.78^{+}	17.75±1.72*
3	25.50±2.42	24.95±2.07^{+}	10.72±1.06*
5	43.58±3.99	31.88±2,15^{*+}	20.51±1.95*
9	39.46±2.67	34.97±2.34^{+}	25.12±2.23*
13	36.97±3.01	34.86±3.33	36.28±2.08
17	24.61±2.22	25.14±1.95	24.47±1.91
21	39.71±3.80	45.42±3.88	49.98±4.28

Note. $p<0.05$: *compared to the control; $^{+}$compared to prednisolone-treated animals.

TABLE 2. Dynamics of Clinical and Laboratory Parameters (*M*±*m*)

Parameter	Artrofoon			Diclophenac		
	baseline	3 months	6 months	baseline	3 months	6 months
Total severity of pain, points	1.80±0.07	1.30±0.07***	1.10±0.05***	1.70±0.09	1.20±0.10***	1.20±0.10***
Morning stiffness, min	134.00±9.85	101.10±7.11***	86.50±5.95***	131.70±13.76	102.50±8.78**	101.30±9.40***
Richie index, points	20.00±0.90	13.90±0.77^{***+}	12.30±0.75^{***++}	22.00±1.11	17.20±1.07***	19.30±1.03***
Number of painful joints	17.40±1.08	14.90±0.93^{***+}	13.60±0.86^{***++}	19.50±1.36	17.80±1.10	17.60±1.22**
Swelling index, points	11.80±1.19	9.00±1.02***	7.10±0.76***	13.90±1.42	9.60±1.11***	10.30±1.46***
Lee index, points	17.00±0.72	15.00±0.68^{***+}	14.20±0.63^{***++}	18.40±0.99	17.40±0.97*	17.20±0.91*
Number of swollen joints	6.70±0.74	5.20±0.53***	4.20±0.45***	8.50±0.99	5.80±0.67***	5.80±0.72***
TNF-a, pg/ml	353.90±38.47	299.80±31.34*	299.70±30.42**	329.00±37.36	326.40±52.22	336.40±49.94
IL-1b, pg/мл	299.50±33.24	272.90±32.44	254.60±29.35*	256.30±45.16	199.8±32.1	255.60±28.74

Note. $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared to the baseline level; $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ compared to Diclophenac-treated patients.

The time of attaining the maximum concentration of IL-1β in blood plasma of control mice was 5 days. C_{max} of IL-1β in control animals was 17.91-fold higher than in intact mice ($p<0.05$). The concentration of IL-1β in blood plasma of control mice decreased slowly. The concentration of IL-1β in blood plasma of control mice was 7.73 times higher than in intact animals on day 21 ($p<0.05$). The test drugs significantly decreased the concentration of IL-1β in blood plasma of animals compared to control mice (days 1-21, $p<0.05$). The concentration of IL-1β in blood plasma of prednisolone-treated mice was higher than in animals of the Artrofoon group.

In all periods of the study, the concentration of IL-1β in supernatants of peritoneal macrophages from control mice was above the baseline level ($p<0.05$). IL-1β production reached maximum 3 h after CIA. C_{max} of IL-1β in supernatants surpassed the baseline level by more than 20 times. Artrofoon contributed to a significant decrease in IL-1β production by peritoneal macrophages of mice compared to control animals (3 h and 3, 9, and 21 days after the start of study; $p<0.05$). Prednisolone also decreased IL-1β production by peritoneal macrophages on days 1-13. It should be emphasized that IL-1β production by peritoneal macrophages from Artrofoon-treated mice was completely suppressed on days 3 and 13.

As differentiated from Artrofoon, prednisolone withdrawal was accompanied by an increase in plasma concentrations of TNF-α and IL-1β. Cytokine production by peritoneal macrophages in these mice increased to the control level.

Clinical manifestations of RA in patients of both groups decreased after 6-month therapy. However, Artrofoon produced a more potent effect. Clinical improvement in Artrofoon-treated patients was accompanied by a decrease in the concentration of proinflammatory cytokines in blood plasma. The concentration of TNF-α ($p<0.05$) and IL-1β decreased in 72.2% patients. The concentration of proinflammatory cytokines in Diclophenac-treated patients did not decrease, but tended to increase (Table 2).

Our results indicate that Artrofoon produces an antiinflammatory effect on mice with CIA and reduces clinical signs of inflammation in patients with RA. The effect of Artrofoon is partly related to a decrease in systemic production of proinflammatory cytokines TNF-α and IL-1β. Hence, this drug produces the pathogenetic effect.

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