Inflammatory Degeneration of Joint Tissue in Adjuvant Arthritis after Intraarticular Treatment with the Mixture of Silver Drug and Nicotinic Acid

V. A. Mitrofanov, N. M. Ovchinnikova, S. V. Belova, M. V. Fedotova, and E. V. Gladkova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 12, pp. 655-657, December, 2005
Original article submitted July 27, 2005

Combination intraarticular administration of Poviargol (0.5 mg/kg) and nicotinic acid (1.0 mg/kg) reduced symptoms of local and general inflammation in rats with adjuvant arthritis. We revealed a decrease in morphological signs of inflammatory degeneration of joint tissue and reduction of metabolic disorders.

Key Words: *arthritis; degeneration; Poviargol; nicotinic acid*

The development of methods for local therapy of autoimmune arthritis is an urgent problem. Intraarticular administration of glucocorticoids and nonsteroid drugs often causes serious complications, which restricts their use in clinical practice [7]. It is important to evaluate therapeutic activity of silver drugs possessing antibacterial [2], viricidal, and immunomodulatory properties. It is pathogenetically substantiated to study the effect of a Russian silver drug Poviargol (PA) on inflammatory degeneration of connective tissue during experimental adjuvant arthritis (AdA). Clinical and morphological signs of AdA are similar to those of rheumatoid arthritis in humans [4]. PA includes a chondroprotective agent polyvinylpyrrolidone, which justifies the intraarticular route of treatment with this preparation [6]. PA is used in combination with nicotinic acid (NA) that normalizes metabolism in tissues and increases the oxidation-reduction potential in cells.

Here we studied the effect of intraarticular treatment with a mixture of PA and NA on inflammatory degeneration of joint tissue in animals with AdA.

Saratov Research Institute of Traumatology and Orthopedics, Russian Public Health Agency. ***Address for correspondennce:*** sarniito@yandex.ru. V. A. Mitrofanov

MATERIALS AND METHODS

Experiments were performed on 40 adult male rats weighing 200-230 g. The animals were kept in a vivarium under standard conditions. AdA was produced by administration of complete Freund's adjuvant (0.1 ml) into the hindlimb pad. The animals were divided into 5 groups (8 rats per group): control group 1, healthy rats; control group 2, untreated rats with AdA; group 3, treatment with 0.25% solution of PA (0.25 mg/kg) and NA; group 4, treatment with 0.5% solution of PA (0.5 mg/kg) and NA; and group 5, treatment with 1.0% solution of PA (1.0 mg/kg) and NA.

Therapy was started on the 3rd day after adjuvant administration. This period correspond to the appearance of clinical signs of arthritis (edema, hyperemia, painfulness, and limitation of movements). PA powder was added to an ampoule with 1.0% solution of NA to obtain 0.25, 0.5, and 1.0% solution of PA. After dissolution of the powder, this mixture (0.02 ml) was injected into the knee joint using a syringe. The dose of NA was 1.0 mg/kg. Injections were performed every 4th day for 16

0007-4888/05/1406-0702

days. The animals were decapitated under ether anesthesia on the next day after the last injection. Experiments were performed with samples of the blood, articular cartilage, and synovial membrane. Signs of local inflammation were clinically evaluated. Histological examination was performed with micropreparations of the synovial membrane and articular cartilage (hematoxylin and eosin or toluidine blue staining). Signs of damage were subjected to a semiquantitative analysis and expressed in points [8]. A cytometric study of the synovial membrane and articular cartilage was performed using an Avtandilov grid [1]. Laboratory tests for metabolic disorders included the measurement of blood malonic dialdehyde level (MDA). This parameter serves as a marker of the intensity of lipid peroxidation (LPO) [5]. Plasma glycosaminoglycan concentration (marker of connective tissue destruction) was determined by the contents of uronic acids and hexoses [3]. The results were analyzed by Student's *t* test.

RESULTS

Clinical signs of joint inflammation were revealed in animals of control group 2 (edema, hyperemia, pain reaction during palpation, and limitation of movements). The articular circumference (6.38±0.09 cm) and skin temperature (37.39±0.09°C) in these rats were much higher than in healthy animals (4.15±0.03 cm and 34.06±0.04°C, respectively, $p<0.001$). In treated rats of groups 3, 4, and 5 we revealed a significant decrease in the articular circumference (4.81±0.12, 4.36±0.03, and 4.37±0.04 cm, respectively, $p<0.001$) and skin temperature (35.20±0.12, 34.90±0.09, and 34.98±0.09°C, respectively, $p<0.001$) compared to animals of control group 2. The test parameters in group 3 rats were lower than in animals of groups 4 and 5 ($p<0.01$).

Microscopic examination revealed focal proliferation of synoviocytes, areas of surface necrosis, and fibrin deposits in the synovial membrane of group 2 rats. The major sign of damage was diffuse lymphoid, histiocytic, and plasmocytic infiltration of the subintimal layer. We compared cytometric characteristics of the synovial membrane in rats of groups 3, 4, and 5 (Table 1). Therapy significantly decreased the number of inflammatory cells and increased the count of fibroblasts.

The degree of cell infiltration in group 3 rats (treatment with 0.25% PA) was higher than in animals of groups 4 and 5. The major signs of damage to the synovial membrane in treated rats were focal fibrosis of the stroma and proliferation of fibroblasts. Degenerative changes in the articular cartilage included focal erosion of the outer layer, presence of cell-free regions, decrease in the number of chondrocytes, and degeneration of some chondrocytes. Morphological study showed that the total joint damage score in group 2 rats is much higher compared to other animals (Table 2). The total damage score in animals of groups 4 and 5 was much lower than in group 2 rats.

Blood analysis showed that MDA concentration in group 2 rats was 2.27 times higher than in healthy animals (6.50±0.42 and 2.86±0.10 μmol/liter, respectively, $p<0.001$), which attests to activation of LPO in group 2 rats. Blood MDA concentration in rats of groups 3, 4, and 5 (3.68±0.12, 3.50±0.14, and 3.30±0.10 μmol/liter, respectively) was lower than in group 2 animals. The data on morphologically verified destruction of the joint tissue in all

TABLE 1. Cytometric Characteristics of the Synovial Membrane in Knee Joints of Rats with AdA (%, *M*±*m*, *n*=8)

Group	Fibroblasts	Histiocytes	Lymphocytes	Plasma cells
2	16.79±0.86	28.46±0.24	36.52±0.22	18.23±0.96
3	26.65±1.13*	25.36±0.34*	32.78±0.35*	15.01±0.32*
4	43.58±0.97*+	23.64±0.26*+	22.53±0.24*+	10.25±0.68*+
5	43.09±0.68*+	23.85±0.44*+	23.31±0.68*+	9.86±0.69*+

Note. $p<0.001$: *compared to group 2; +compared to group 3.

TABLE 2. Morphological Parameters (Total Score) of Damage to Joint Tissue in Experimental Animals (*M*±*m*, *n*=8)

Tissue	Group 2	Group 3	Group 4	Group 5
Synovial membrane	6.98±0.52	4.98±0.25*+	3.45±0.12*+	3.68±0.11*+
Articular cartilage	8.41±0.35	5.38±0.11*	3.68±0.11*+	4.03±0.17*+

Note. $p<0.001$: *compared to group 2; +compared to group 3.

TABLE 3. Plasma Glycosaminoglycan Concentration in Experimental Animals ($M \pm m$, Content of Uronic Acids and Hexoses, $g \times 10^{-2}$/liter)

Group	Uronic acids	Hexoses
1 (n=6)	1.39±0.04	3.58±0.50
2 (n=6)	4.25±0.25*	5.93±0.22*
3 (n=7)	3.28±0.25*+	5.76±0.44**
4 (n=5)	2.23±0.24**+++o	3.31±0.47***+++o
5 (n=8)	2.65±0.35**++	3.52±0.14***++o

Note. *p<0.001, **p<0.01, and ***p<0.02 compared to group 1; +p<0.05, ++p<0.01, and +++p<0.001 compared to group 2; °p<0.01 compared to group 3.

animals with experimental AdA was consistent with the increase in plasma glycosaminoglycan concentration (Table 3).

Our results show that intraarticular administration of PA and NA mixture produces a complex effect on the pathogenetic mechanisms of AdA. Immunomodulatory activity of silver clusters prevents hyperstimulation of immunocompetent cells in the synovial membrane. Polyvinylpyrrolidone entering the composition of PA protects the cartilage from degeneration during inflammation. NA improves tissue reparation by reducing DNA damage. These properties of the test mixture were confirmed by the results of morphological (reduction of degeneration in joint tissue) and biochemical (decrease in the concentrations of MDA and glycosaminoglycans in the blood from treated rats) tests. It should be emphasized that the mixture containing 0.25% PA did not produce a strong therapeutic effect.

Intraarticular administration of 0.5% PA and NA alleviates the signs of local joint inflammation, prevents degeneration of joint tissue, and decreases the severity of metabolic disorders. These changes determine a therapeutic effect of the test mixture during AdA.

REFERENCES

1. G. G. Avtandilov, *Problems of Pathogenesis and Pathoanatomical Diagnostics of Diseases in Terms of Morphometry* [in Russian], Moscow (1984).
2. A. M. Gnetnev, V. A. Burmistrov, and P. P. Rodionov, *Polyethylene Oxide Gel - 1500 Geko* [in Russian], Novosibirsk, Saratov, Pyatigorsk (2001), pp. 102-111.
3. E. V. Karyakina and D. V. Kosyagin, *Lab. Delo*, No. 10, 591-593 (1982).
4. T. N. Kop'eva, *Pathology of Rheumatoid Arthritis* [in Russian], Moscow (1980).
5. E. N. Korobeinikova, *Lab. Delo*, No. 7, 8-10 (1989).
6. A. A. Matulis, I. G. Dadonene, and R. I. Grigene, *Ter. Arkhiv*, No. 5, 95-98 (1990).
7. N. V. Chichasova, *Rus. Med. Zh*., Nos. 7-8, 286-288 (2001).
8. C. Colombo, M. Butler, E. O'Byrne, *et al*., *Arthritis Rheum*., **26**, No. 7, 875-886 (1983).