The Connective Tissue in Local Therapy of Experimental Arthritis with Miacalcic

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Effects of local therapy with miacalcic on the metabolism in articular connective tissue structures under conditions of experimental arthritis are shown. An effect modifying articular tissue metabolism and an antiinflammatory effect preventing the development of inflammatory destructive processes in rabbit articular tissues were detected.

Key Words: *experimental arthritis; connective tissue; miacalcic*

Despite recent progress in the treatment of rheumatoid arthritis (RA), drug therapy of this disease remains an important problem of medicine [5].

The leading mechanisms of RA development are disorders in connective tissue metabolism and pronounced inflammatory destructive processes in connective tissue structures. Osteoporosis is a characteristic manifestation of RA [2-4].

Salmon calcitonin (miacalcic) modifying bone tissue metabolism is widely used for the treatment of patients with RA complicated by osteoporosis. Miacalcic is prescribed as a part of combined therapy for RA as nasal spray, subcutaneous and intramuscular injections [1,6-8]. The efficiency of local application of miacalcic in RA is not yet proven.

We evaluated the effects of intraarticular administration of miacalcic on connective tissue structures of involved joints in rabbits with experimental RA.

MATERIALS AND METHODS

The study was carried out on 52 adult Russian Chinchilla rabbits (3.0-3.5 kg), RA was modeled in 42 animals. The rabbits were injected with miacalcic intraartciularly in a dose of 0.1 ml once a week, 5 injections per course (experimental group, n=32) or 0.9% NaCl (control group, n=10).

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The antigenic adjuvant model of experimental arthritis was formed using complete Freund's adjuvant, containing heat-inactivated mycobacteria (dry BCG vaccine) in mineral oil; ovalbumin served as the antigen. Experimental rabbits were intracutaneously injected with 4 mg ovalbumin in 1 ml complete Freund's adjuvant into the right knee joint (KJ); the injection was repeated after 14 days. Five days after repeated immunization, the resolving dose of the antigen (5 mg ovalbumin in 1 ml 0.9% NaCl) was injected into the right (experimental) KJ and 1 ml 0.9% NaCl into the left KJ.

Clinical laboratory studies were carried out in all animals before immunization, on day 20 after immunization (when RA model was already formed), and after the course of treatment.

The status of animals was evaluated by clinical methods: examination, evaluation of rabbit behavior and activity, weighing. The circumferences of intact and involved KJ were measured and their mobility was evaluated. Distant thermography of KJ was used for objective verification of arthritis development. Connective tissue metabolism was evaluated by the total content of glycosaminoglycans (GAG) and content of their fractions in the serum. Mineral metabolism was studied by measurements of total (Fluitest Ca-CPC, Biocon) and ionized Ca^{2+} (Ciba-Corning M 634 automated analyzer), inorganic phosphorus (Phosphorus UV FS, DiaSys), alkaline phosphatase (Alkaline phospha-

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tase Fs DGKS, DiaSys) in the serum by the standard methods.

The animals were sacrificed by intravenous injection of 1% promedol (1 ml/kg) into the marginal ear vein, after which histomorphological study of KJ tissues (synovial membrane, meniscus, articular cartilage, subchondral bone) and semiquantitative (score) evaluation of signs of their involvement were carried out.

The data were statistically processed using Student's *t* test.

RESULTS

Twenty days after immunization the animals were much more inert than intact animals. All immunized rabbits developed pronounced RA of the right KJ. Involvement of the KJ manifested in edema, high local temperature, limited mobility, pronounced pain (the animals spared the limb and reacted to pain during palpation of KJ). The maximum increase of the KJ circumference (by 9-12 mm) was observed 3-4 days after the resolving dose of ovalbumin.

On the other hand, thermovision showed pronounced thermal asymmetry of the KJ area in immunized rabbits: the temperature of the involved KJ was 1.0-1.3°C higher than that of intact KJ.

All animals with RA developed a pronounced inflammatory reaction: erythrocyte sedimentation rate 2-fold surpassed the normal (6.5±0.3 mm/h *vs.* 3.1±0.2 mm/h), leukocyte count was increased, and the hemocytogram was shifted "to the left".

Serum level of GAG, evaluated by uronic acids and by hexoses, levels of total and ionized Ca^{2+} and phosphorus increased significantly, while alkaline phosphatase activity decreased in rabbits with RA before treatment (Table 1). Mineral metabolism disorders manifested in elevation of total and ionized Ca^{2+}, phosphorus content, and reduction of alkaline phosphatase activity in the blood of animals with RA (Table 1).

Histomorphometrical analysis also indicated inflammatory cell infiltration and degradation of connective tissue KJ.

Positive shifts in clinical laboratory values were observed in experimental animals after treatment. Statistically significant positive shifts in the hemocytogram were observed: erythrocyte sedimentation rate decreased, erythrocyte count and hemoglobin content increased, total leukocyte count (stab and segmented forms) decreased.

In controls, the connective tissue metabolism and mineral metabolism values progressively increased (Table 1). Serum levels of GAG, phosphorus, total and ionized Ca^{2+} were significantly ($p<0.05$) higher than in animals treated with miacalcic. Alkaline phosphatase activity was much lower than in the group of treated rabbits, although this difference was insignificant. Hence, inflammatory destructive processes in the connective tissue still progressed in control animals.

The efficiency of correction was evaluated by macro- and microscopic study of experimental KJ tissues, including the paraarticular tissues and subchondral bone. Macroscopic examination showed significant pathological changes in the studied tissues of all control animals: the paraarticular tissues were thickened and loosened, the articular cartilage had a dull degenerative surface at an appreciable length. Bone growth in the form of osteophytes at the edges of the external tibial condyles was seen in some rabbits.

In the experimental group slight thickening of paraarticular tissue was detected in only two rabbits; the articular cartilage in these animals had small rough areas in the marginal condylar zones. No pathological changes in the bone tissue were detected.

TABLE 1. Metabolism and Mineral Exchange in the Blood of Intact Rabbits and Rabbits with Experimental RA ($M\pm m$)

Parameter	Intact rabbits (n=10)	Control group (n=10)	Experimental group	
			before therapy (n=32)	after therapy (n=32)
GAG, g×10^{-2}/liter				
by uronic acids	1.23±0.10	3.12±0.14	2.02±0.12*	1.86±0.09**+
by hexoses	1.37±0.03	4.06±0.11	2.71±0.05*	2.08±0.25**+
Ca^{2+}, mmol/liter				
ionized	1.450±0.015	2.47±0.06	2.32±0.16*	1.62±0.02**+
total	2.74±0.06	3.53±0.05	3.48±0.12*	2.91±0.08**+
Inorganic phosphorus, mmol/liter	2.34±0.07	3.68±0.16	3.11±0.19*	2.52±0.27**
Alkaline phosphatase, U/liter	190.00±15.98	151.00±14.72	164.00±12.36*	182.00±11.29

Note. $p<0.05$ compared to: *intact rabbits, **control group, +data before therapy.

TABLE 2. Signs of KJ Tissue Involvement (Score) in Untreated Rabbits and after Miacalcic Treatment ($M \pm m$)

Tissue	Control group (n=10)	Miacalcic therapy (n=32)
Paraarticular	5.40±0.11	2.17±0.11*
Synovial membrane	6.13±0.72	3.68±0.22*
Articular cartilage	8.12±0.28	3.47±0.14*

Note. *p<0.001 compared to the control group.

Comparative analysis of the results of morphometrical studies of articular tissues confirmed these data (Table 2).

Hence, local miacalcic therapy produced an antiinflammatory effect and improved mineral metabolism in the bone tissue and metabolic processes in the matrix of the KJ connective tissue elements, which promoted arrest of inflammatory destructive processes.

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