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New possibilites for treatment of chronic synovitis in rabbit experimental arthritis

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Chronic synovitis presenting in patients with systemic inflammatory joint diseases acts aggressively on all structural elements of the joint, especially on articular cartilage.

The purpose of this study was to confirm the remote effectiveness of low concentrations of intraarticularly injected sclerosants (sol. 0.1% Papaini and 1% Dioxidini) in a long distance of time.

Metods: Experimental chronic arthritis was induced by the method of Dumone and Glynn modified by us. Histopathological changes in synovium and hyaline cartilage of rabbit knee joint induced by intraarticular injections of sclerosants has been described by us in detail in previous articles.

Results: All sclerosants induced various degrees of fibrosis and fibrosclerosis in subsynovial stroma in the synovial layer and provoked a deep destruction of joint hyaline cartilage. Some sclerosants that induced synoviorthesis were found to have a minimal destructive ability on hyaline cartilage and by spreading of proliferated mesenchymal cells from the synovial/cartilage junction to stimulate the restoration of hyaline cartilage.

Conclusion: Sol. Papaini 0.1% and sol. Dioxidini 1% are promising candidates for the intraarticular treatment of chronic synovitis.

Key words: experimental arthritis, sclerosants (sol. Papaini 0.1% and sol. Dioxidini 1%), synoviorthesis, cartilage

INTRODUCTION

Systemic inflammatory joint diseases presenting by chronic effusion are wearisome not only for the patients but also deepen the destructive changes in all structural elements of the joint, especially in hyaline cartilage, by releasing inflammatory mediators from the inflamed synovium.

To suppress chronic inflammation of synovium, various methods of intraarticular treatment have been used, such as corticosteroids (1), radioactive and chemical synoviorthesis (2), hyaluronic acid, diclophenac and opioid analgesia solutions (3) and others, as well as surgical synovectomy (4).

However, beside the desirable effect on synovial inflammation, all these sclerosing agents induce direct destruction of hyaline cartilage and other joint structures and lead to development of secondary arthrosis.

Recently the sclerosing agents for sclerotherapy of hepatic and kidney varicocele, leg varices and for pleural cavity obliteration in chronic pleuritis have been described (5–7). We used them for the intraarticular treatment of rabbits with chronic arthritis at high and low concentrations and at different time of exposure in joint cavity in several experimental studies.

Evident suppression of inflammation, induction of fibrosis and fibrosclerosis of subsynovial stroma and the wall of blood vessels by sclerotherapy was found. Therefore, independently of the type of sclerosant and its concentrations or the time of exposure of a joint cavity, a destruction of hyaline cartilage was observed (8–10).

Only Dioxidini and Papaini induced a sufficient fibrosclerosis of synovial membrane, together with some destruction of articular cartilage, and stimulated the spreading of proliferating mesenchymal cells from synovial / cartilage junction into the middle layer of cartilage and thus exibited the ability to restore the cartilage.

The purpose of this study was to confirm the effectiveness of low concentrations of these sclerosants (sol. 0.1% Papaini and 1% Dioxidini) in a long perspective of time after intraarticular injections.

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MATERIALS AND METHODS

Thirty rabbits, the mean age 2.5 months, weighing 2200–2600 g, kept under uniform conditions, on similar meal and water were used in this study. All procedures were performed at the Institute of Experimental and Clinical Medicine. The study was performed with a due regard to the current laws of Lithuania on animal welfare, and the protocol for the study was approved by the Lithuanian Ethical Committee for Laboratory Animal Usage.

Experimental chronic arthritis was induced by the Dumone and Glynn method (11) modified by us. For induction of arthritis, accelerated cycles of immunisation were used. Thirty rabbits were immunised with synovial fluid (SF) obtained from a patient with active seropositive rheumatoid arthritis (RA). Sensibilization with a mixture of the same SF homogenised with Freund's adjuvant (10:1) containing 2 mg of *Mycobacterium tuberculosis* was used, ten injections 0.1 ml each of the mixture were introduced into both sides of the rabbit's back, and the sensibilization was repeated after two weeks. Ten days following the last sensibilization, active immunisation was started with 1 ml of SF injected intraarticularly into both knee joints of rabbits 3 times per week.

Ten days following the last immunization of rabbits, active immune synovitis developed in all 30 rabbits. The rabbits were devided into two groups and 1 ml of intraarticular injection of sol. Papaini 0.1% (group I) and 1 ml sol. Dioxidini 1% (group II) was introduced to the rigt knee joint. The left knee joint with injected with 1 ml of sterile physiologic saline and served as control.

The rabbits were sacrificed by an overdosage of 25 mg of natrium thiopental anaesthesia following 4 (6 animals), 6 (6 animals) and 8 (8 animals) weeks of treatment. A macroscopic evaluation of joint structures after arthrotomy was done. For microscopic investigation, synovial membrane and articular cartilage from both knee joints (right as experimental and left as control) were obtained. Histopathologic changes in the control and experimental groups were evaluated by grading the pathological changes in the synovial membrane and articular cartilage of the left knee joint after 4, 6 and 8 weeks of intraarticular injections.

The material was fixed in an ethanol-formaldehyde (9:1) fixative and embedded into paraffin. Hematoxylin-eosine, toluidine blue pH 4.5, safranin O, van Giesone, methyl green-pyronine according to Bracchet and periodic acid – Schiff (PAS) reaction were used for staining the tissue slices.

RESULTS

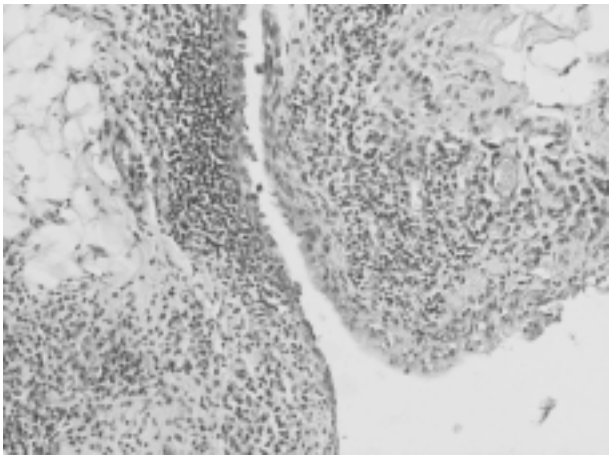
Ten days after the last immunisation, active immune synovitis developed. The mean volume of the joints increased by 8 mm and a fluctuating defiguration was evident.

The strongest histopathological signs in the synovial membrane of control joints was hyperplasia of synoviocytes A (up to 12 layers), villous proliferation, subsynovial edema, γ-metachromasy and subsynovium inflammation as well as vasculitis. Venular stasis of synovium proved the development of chronic synovitis (Figure, A).

Destructive changes in the articular cartilage of the left knee joint were also obviously induced by

Table 1. **Grading of histopathological changes in the synovium of rabbit knee joint after intraarticular injections of Papain 0.1% and Dioxydin 1% solutions in EA**

	EA			EA + Papain 0.1%			EA + Dioxidin 1%		
	4 w	6 w	8 w	4 w	6 w	8 w	4 w	6 w	8 w
Villi proliferation	16	13	0.75	2.3	2.3	1.5	2.6	2.6	1.0
Layers of A-synoviocytes	3.6	0.6	0.25	5.3	3.0	2.5	2.6	3.2	3.15
Focal edema	2.3	2.3	0.5	1.6	1.6	1.0	3.0	2.6	1.25
Focal γ-methachromasy	1.6	2.3	0.5	1.3	2.16	1.0	2.8	2.5	1.43
Fibrinoid	0	0.3	0.5	1.0	1.0	0.75	1.0	2.6	0.25
Inflammation (scores): focal	0.3	0.3	1.25	0.6	1.3	1.0	0.6	0.6	
follicular	0	0	0	0	0	0	0.3	0.6	
diffuse	1.3	2.0	0.75	1.3	1.6	0.75	1.6	2.0	
Inflammatory necrobiosis	1.0	1.3	1.5	1.0	1.0	2.25	1.0	0.6	
Vasculitis	0	0	0.75	0.6	0.3	0	0.6	1.0	
Perivasculitis	0.6	0	0	0	0	0.5	0.6	0.6	
Angiomatosis	1.3	0	0	0.6	0	1.5	1.6	0.6	2.25
Fibrosis	2.3	2.6	2.5	2.3	2.6	3.0	3.0	3.0	3.0
Lipomatosis	2.0	2.0	2.5	2.0	1.3	1.0	1.3	2.6	1.0



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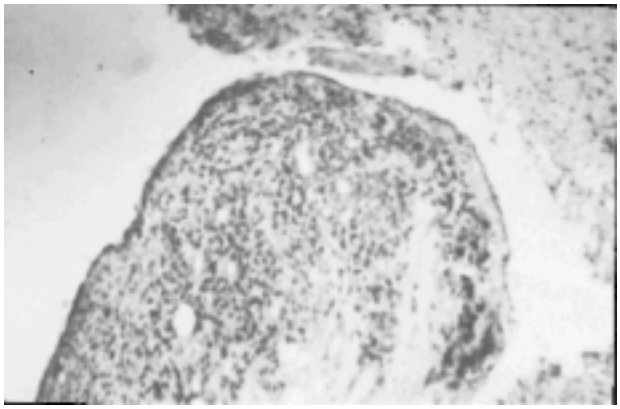

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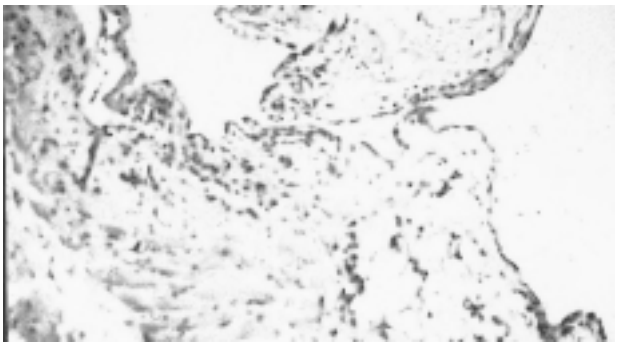

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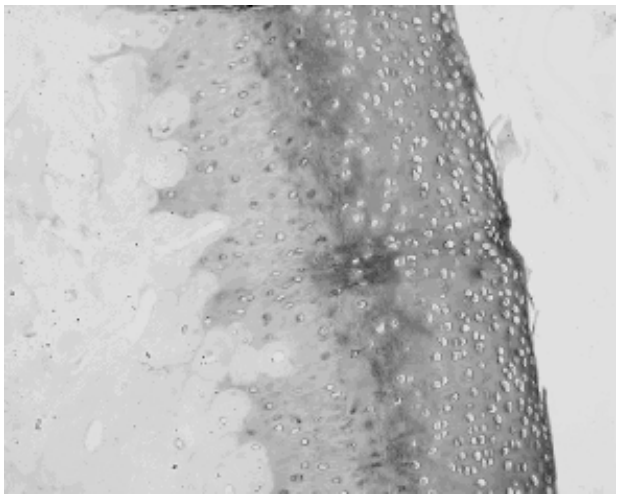

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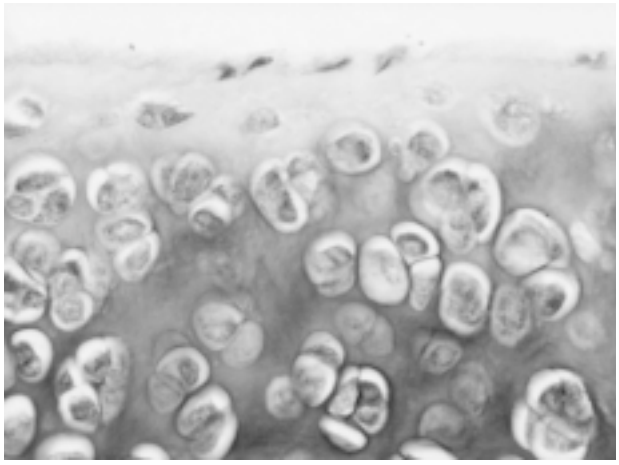

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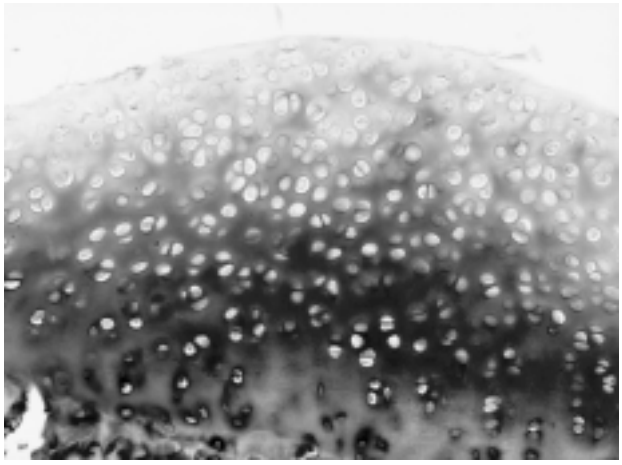

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Figure. Histopathological signs in experimental chronic arthritis: villi proliferation, synoviocytes-A focal hyperplasia, strong inflammatory infiltrations and vasculitis in edematous subsynovium (A). Erosions, decreased PG content and destruction of chondrocytes in all layers of hyaline cartilage (D). Subsynovial edema and slight diminution of inflammatory infiltrations in synovium after intraarticular injection of 0.1% Papaini (B). Prominent synoviorthesis after intraarticular injection of 1% Dioxydini (C). Round, activated with some mitosis of chondrocytes in hyaline cartilage after intraarticular injection of 0.1% Papaini (E). Focal thickening of hyaline cartilage with prominent PG content in lower layers of cartilage after intraarticular injection of 1% Dioxydini (F). Hematoxylin-eosine, methyl green-pyronine, toluidine blue. ×200, 400

synovitis. Erosions, usurations, fissures, inflammatory pannus and atrophy of cartilage were prominent. Metabolic signs such as decreased content of PGs in the middle and deep layers of cartilage were more pronounced in the latest periods of arthritis development and represented disturbances not only of the structure but also of the chondrocyte metabolism (Figure, D).

A summary evaluation of histophatological changes in synovium and in articular cartilage and the possibility to repair hyaline cartilage upon intraarticular treatment with sol. Papaini 0.1% and sol. Dioxidini 1% in chronic synovitis is presented in Tables 1 and 2.

Influence of sol. Papaini 0.1% intraarticular injection on histopathological changes in knee joint synovium of rabbits with EA (Table 1)

Four weeks after injection of sol. Papaini 0.1% to the right knee joint the proliferation of villi was increased, with a focal proliferation of synoviocyte layers (up to 7). An increased fibrinoidal impregnation of the subsynovial connective tissue appeared, but focal diminution of γ-metachromasy was seen too. A moderate increase of inflammation in large foci was found, and small perivasculitis was increased too in comparison to control joints.

Six weeks following the therapy, villi proliferation was increased and more foci of proliferated synoviocyte-A appeared in comparison to the control joint. However, edema and γ-metachromasy of the subsynovial layer were diminished, foci of fibrinoidal impregnation were the same as after 4 weeks, and the vasculitis of small vessels was diminished. Fibrosis and lipomatosis of the subsynovial layer were the same as in the control group and after 4 weeks of sclerotherapy.

Eight weeks post intraarticular therapy, a proliferation of synovial villi was slightly decreased, although more evident than in control joints, and the foci of synoviocyte-A proliferation (up to 4 layers) were increased, as was also subsynovial edema, but the inflammatory component and signs of vasculitis were diminished. The strengthening of subsynovial angiomatosis and fibrosis and some weakening of lipomatosis were found (Figure, B).

Influence of sol. Papaini 0.1% on hyaline cartilage (Table 2)

Four weeks post intraarticular injection of sol. Papaini 0.1% to the right knee joint, some erosion in comparison to control knee joint cartilage was seen, althouh GAGs were washed to the deep and middle zones and edema of these layers became evident. Chondrocytes of the upper layer of cartilage were flat with the picnotic nucleus and desquamated. Clusters of chondrocytes with two nuclei in the middle layer, spreading from the synovial / cartilage junction were seen. Chondrocytes with some signs of destruction were seen, however, in the deep zone prevailed tidily columned chondrocytes, some with condensated chromatin of the nucleus.

Six weeks following intraarticular injection of sol. Papaini 0.1% abudant proteoglycans were observed through all layers of cartilage together with increased edema. Round activated chondrocytes flew farther from the synovial / cartilage junction and were divided not only in the middle but also in the columned layer. Columnated chondrocytes were lined tidily, but foci of chondrocytes with condensated nuclei were observed.

Eight weeks post treatment more erosions on the surface of cartilage were found. In the upper layer of cartilage prevailed round activated chondrocytes with two nuclei, and the picnosis of their nuclei was episodical. In the middle layer of cartilage, besides round, activated chondrocytes, some signs of chondrocyte destruction in the deep layer as well as tidily arranged columnal chondrocytes were seen (Figure, E).

Table 2. **Grading histological changes in hyaline cartilage of rabbit knee joint 4, 6 and 8 weeks after intraarticular injections of 0.1% Papaini and 1% Dioxydini solutions in EA**

Histopathological changes		EA			EA + Papain 0.1%			EA + Dioxydin 1%		
		4 w	6 w	8 w	4 w	6 w	8 w	4 w	6 w	8 w
Surface of cartilage	Pannus	0	0	0	0	0.3	0	0	1.3	0
	Erosions	1.3	0.6	1.87	1.3	1.6	2.25	0.6	2.0	1.25
	Usures	0	0	1.0	0	1.0	2.25	0.6	0.6	0
	Fissures	0	0.3	0.5	0.3	0.3	0.5	0	0	0
Chondrocytes /GAG	I	1/1.6	1/2.0		0.9/1.6	1/2.75	1.25/0.75	0.9/0	0.9/1.16	1.0/2.25
	II	0.5/1	1.2/2.0		2.0/1	1/2.75	1/1.12	0.6//1.3	1.0/1.3	1.0/2.37
	III	1.5/1.6	1.08		0.9/1.6	0.9/2.75	0.75/1.0	0.3/2.0	0.9/1.83	1.0/2.37
Atrophy	0	1.0	0.75	2.0	1.0	2.75	1.3	1.6	0.25	
Edema	0.3	0	1.0	0	0.3	0	0.6	0.3	1.75	

Influence of intraarticular injection of sol. Dioxidini 1% on histopathological changes of knee joint synovium in rabbits with EA (Table 1)

Four weeks after intraarticular treatment, a distinct proliferation of villi with somewhat more layers of proliferated A-synoviocytes, a stronger subsynovial edema and γ-metachromasy, more focal and diffuse inflammatory infiltrations with some necrobiosis of inflammatory foci were observed. A diffuse spreading of histiocytes, some signs of small perivasculitis and even signs of vasculitis (with damaged walls of blood vessels) appeared. Foci of angiomatosis and pronounced fibrosis of subsynovium were visible.

Six weeks post intraarticular injection, proliferated villi on the surface of synovium were at the same level, A-synoviocytes were up to 3–4 layers. Intensification of subsynovium γ-metachromasia and fibrinoidal infiltrations on the foci (0.3–2.6) were seen. Some large foci of follicularly arranged inflammatory infiltrations proceeded by a diffuse spreading of histiocytes, but smaller foci of vasculitis became evident. Subsynovial fibrosis (up to 3 scores) and lipomatosis (up to 2.6 scores) were increased.

Eight weeks post intraarticular treatment the synovial surface was covered with fibrin with increased foci of proliferated A-synoviocyte (up to 7 layers) and sites of γ-metachromasy, but the fibrinoid impregnation of the subsynovial layer was less. Unfortunately, some foci of large follicular inflammatory cells appeared again, diffuse infiltration of hystiocyte became stronger, the same level of fibrosis and a slightly decreased lipomatosis were seen (Figure, C).

Influence of intraarticular injection of sol. Dioxidini 1% on histopathological changes of knee joint articular cartilage in EA

Four weeks following intraarticular treatment, erosions on the surface of cartilage, focal sites of cartilage edema and atrophy appeared. Proteoglycans were washed to the middle and deep zones of the cartilage. Instead of tidily arranged columnal chondrocytes, in control joints hyaline cartilage, chondrocytes with picnotic nuclei (up to 0.3 points) beside normal cells were seen.

Six weeks post intraarticular treatment, fibrin, erosions and usurations, inflammatory pannus on cartilage surface appeared and atrophy of cartilage developed alongside the diminution of proteoglycans in all layers. However, beside flat and desquamated chondrocytes in the upper layer, round and activated chondrocytes were seen. The middle layer of cartilage, making 7–8 lines of chondrocytes, was grouped by multicellular clusters of activated chondrocytes, which spread into the middle zone from the synovial / cartilage junction. In the deep zone prevailed tidily columned chondrocytes; only some of them had condensated chromatin in the nucleus.

Eight weeks post intraarticular treatment more erosions, few solitary usurations of cartilage appeared. A marked diminution of proteoglycans in all layers in comparison to the control knee joint and some signs of cartilage atrophy were found. The chondrocytes became flattened, and condensation of nuclear chromatine in the upper layer of cartilage was seen. Despite the large clusters of chondrocytes in the middle and columned layers, disarranged chondrocytes with condensated chromatine were prevailing and diminution of proteoglycans in all layers of cartilage was seen (Figure, F).

DISCUSSION

Chronic synovitis leads to destructive-degenerative changes of the joint in patients with systemic inflammatory joint diseases. Complex interaction between inflammatory synovium lining as antigen-presenting cells and lymphocytes, synoviocytes and inflammatory cytokines initiates the destruction of joint cartilage and bone and leads to joint deterioration. To avoid joint dysfunction, it is very important to suppress chronic inflammation of the synovial membrane. The contemporary conservative treatment has only a limited beneficial effect on chronic arthritis. For suppression of chronic synovium inflammation, various methods of intraarticular treatment are applied. Besides synoviorthesis, however, such intraarticular treatment causes a direct destruction of cartilage and other joint structures and leads to the development of secondary arthrosis.

Based on this information, we performed several experimental studies on rabbits to check the effect of various sclerosants, introduced intraarticularly for sclerotherapy of chronic synovitis, which are used for scleroterapy of testicular cele, epididymal, renal and hepatic cysts, or oesophageal, haemorrhoidal and leg venous varices (5–7). All sclerosants at high and low concentrations and different time of exposure in joint cavity induced fibrosis and fibrosclerosis of synovial membrane with an evident destruction of cartilage, from erosions and surface usurations up to disarrangement and destruction of chondrocytes by condensation of nucleus chromatine, a decreased synthesis of proteoglycans and atrophy of cartilage.

However, we found that some of the sclerosants tested, which induced distinct synoviorthesis, had a minimal destructive effect on hyaline cartilage, and therefore a tendency to a gradual restoration of cartilage structure and metabolism was found.

We tried to confirm the ability of sol. Papaini 0.1% and sol. Dioxidini 1% to induce synoviorthesis and their potency to restore articular hialine cartilage.

At the end (8 weeks) of experiment only few erosions on the surface of cartilage were found

and active chondrocytes with two nuclei in the upper layer dominated, with a very compactly arranged upper sprout of chondrocytes in one case, after intraarticular injection of sol. Papaini 0.1%. Along with the round, activated chondrocytes, in the middle layer of cartilage tidily arranged columnar chondrocytes with some signs of destruction were seen.

Evidently sol. Papaini 0.1% induced focal activation and spreading of mesenchymal cells from the synovium / cartilage junction into the upper and middle zones and took part in the restoration of the structure and metabolism of cartilage by active production of proteoglycans.

The fibrosing capacity of sol. Papaini on inflammatory synovium was evident and deep. The proliferation of synovial villi was reduced, necrobiosis and desquamation of A-synoviocytes as well as fibrosis of vascular components and subsynovium were evident.

At the end of experiment it was stated that sol. Dioxidini had a transitory antiproliferative and antiinflammatory effect leading to a slight and superficial fibrosclerosis of synovium. Besides, insufficient synoviorthesis activation of chondrocytes in the middle zone together with a focal thickening of cartilage were seen, whereas after intraarticular injection of sol. Papaini an evident accumulation of young chondrocytes in the middle zone of cartilage by spreading the proliferated mesenchymal cells from the synovium / cartilage junction and the thickening of the entire cartilage were observed, together with an increased content of proteoglycans, indicating a certain restoration of the cartilage.

Furthermore, neither subjective nor objective complications have been noted under the effect of these sclerosing agents and synoviorthesis technique over the last four years.

Based on these experimental studies we conclude that sol. Papaini 0.1% and sol. Dioxidini 1% are promising candidates for the intraarticular treatment of chronic synovitis in systemic rheumatic joint diseases. These results suggest that sol. Papaini 0.1% as a sclerosant has a great potency as the cheapest drug to be used by intraarticular injections for joint hyaline cartilage reparation.

However, numerous obstacles should be overcome before introducing sol. Papaini 0.1% and sol. Dioxidini 1% for intraarticular treatment in humans.

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References

1. Papacrhiston G, Anagnostan S, Katsorhri T. The effect of intraarticular hydrocortisone injection on the articular cartilage of rabbits. Acta Orthop Scand Suppl 1997; 275: 132–4.
2. De Vargas AF, Fernandez-Palazzi F. Cytogenic studies in patients with haemophilic hemarthrosis treatment by Au-198, Rh-186 and Y-90 radioactive synoviorthesis. J Paed Orthopaed Part B 2000; 9(1): 52–4.
3. Gøurkan Y, Kiliøckan L, Buluc L, Mønezzinogles S, Toker K. Effects of diclofenac and intraarticular morphine (bupivacaine) on postarthroscopic pain control. Minerva Anestesiol 1999; 39: 741–5.
4. Mohler DG, Kessler BD. Open synovectomy with cryosurgical adjuvant for treatment of diffuse pigmented villonodular synovitis of the knee. Bull Hosp Jt Dis 2000; 59(2): 99–105.
5. Dachlin L, Tisunder B, Kapstad L. Comparison of polidocanol in sclerotherapy of testicular hydrocele and epidydimal cyst. Br J Urol 1997; 80(3): 468–71.
6. Zimmet SE. Treatment of varicose and teleangiectatic leg veins with hypertonic saline (letter, comments). J Dermatol Oncol 1990; 16(9): 876–7.
7. El-Diarty TA, Shokeir A, Tawfeck HA et al. Ethanol sclerotherapy for symptomatic renal cysts. J Enurol 1995; 9(3): 273–6.
8. Staponas A, Gražienė V. Screening of sclerosing agents introduced intraarticularly for synoviorthesis in experimental chronic synovitis. A preliminary report. Acta Medica Lituanica 2000; 7: 128–30.
9. Staponas A, Gražienė V. Screening of sclerosing agents introduced intraarticularly for synoviorthesis in experimental chronic synovitis. A second report. Acta Medica Lituanica 2001; 8: 193–7.
10. Staponas A, Gražienė V. Screening of sclerosing agents introduced intraarticularly for synoviorthesis by different expositions in joint cavity in experimental chronic synovitis. A third report. Acta Medica Lituanica 2002; 9(4): 261–8.
11. Dumonde DC, Glynn LE. The production of arthritis in rabbit leg by immunological reactions to fibrin. Br J Exp Pathol; 43: 373–82.

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NAUJOS TRIUŠIŲ CHRONINIO SINOVITO GYDYMO GALIMYBĖS

S a n t r a u k a

Sergant sisteminėmis uždegiminėmis sąnarių ligomis chroninis sinovitas sukelia ryškius destrukcinius pokyčius visuose sąnario elementuose, ypač hialininėje kremzlėje. Šio darbo tikslas – ištirti sklerozuojančių vaistų poveikį chroninio sinovito eigai.

Metodai: Eksperimentinis chroninis sinovitas triušiams buvo sukeliamas pagal mūsų modifikuotą Dumone ir Glynno metodiką. Histopatologiniai pokyčiai sinoviniame dangale ir hialininėje kremzlėje įvedus sklerozantus į sąnarį autorių buvo detaliai aprašyti ankstesniuose straipsniuose [8, 9, 10].

Rezultatai: Šiais eksperimentais nustatyta, kad visi naudoti sklerozantai sukelia didesnę ar mažesnę sinovinio dangalo fibrozę, taip pat gilius destrukcinius pokyčius hialininėje kremzlėje.

Kartu pastebėta, kad sol. Papaini 0,1% ir sol. Dioksidini 1% ne tik sukelia sinoviortezę, bet ir stimuliuoja jaunų chondrocitų priplūdimą iš kremzlės sinovijos jungties, iš dalies atstato hialininės kremzlės struktūrą ir metabolizmą.

Išvados: Remdamiesi šių eksperimentų histologinio tyrimo rezultatais, manome, kad sol. Papaini 0,1% ir sol. Dioksidini 1% gali būti taikomi chroniniam sinovitui gydyti.

Raktažodžiai: eksperimentinis artritas, sklerozantai (sol. Papaini 0,1% ir sol. Dioksidini 1%), sinoviortezė, kremzlė