Screening of Sclerosing Agents Introduced Intraarticularly for Synoviorthesis in Experimental Chronic Synovitis. A Second Report

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The aim of the study was to evaluate the effectiveness of reduced concentrations of preliminary selected sclerosing agents (1) having a minimal destructive effect on the structure and metabolism of articular cartilage for the intraarticular treatment of chronic synovitis in rabbits. High doses of sclerosants introduced intraarticularly in a preliminary screening study, together with a sclerosing effect of the inflammatory synovial layer, induced distinct destructive changes in articular cartilage. In a second study chronic synovitis was induced in 30 rabbits by a modified method of Dumonde and Glynn described in a preliminary report (1). The rabbits were sacrificed 1, 2, 3, 4 and 5 weeks after intraarticular injection of the sclerosant solution. Such a sequence of study permits to evaluate the dynamics of the development of histopathological changes induced by the sclerosing solution in the synovial layer and articular cartilage.

The histopathological data on the synovial layer and articular cartilage in the second experimental study with intraarticular injections of reduced concentrations of sclerosing solutions in chronic arthritis were almost similar to the results of the preliminary study when high concentrations of sclerosants were used. In the future experimental study we would try to use a different time of introducing the sclerosants into a joint cavity, because the sclerosant concentration did not differ by its influence on the destructive consequences in the articular cartilage.

Key words: experimental arthritis, sclerosants, synoviorthesis

INTRODUCTION

Until now the treatment of chronic synovitis remains the main unsolved problem in rheumatology. Despite conservative treatment with aggressive immunosuppressive drugs, rather often exudation remains in one or more joints of patients with chronic synovitis. This is not only wearisome for the patients, but also deepening the destructive changes in joint hyaline cartilage by released inflammatory mediators from inflamed synovium. In those cases the intraarticular treatment by injections of dexametasone, cortivasole, triamcinolon, hydrocortison and other types of corticosteroids (2, 3) as well as solutions of diclophenac and opioid analgesia (4), hyaluronic acid (5), radioactive and chemical synoviorthese (6, 7) and other kinds of treatments are used. Along with suppressing chronic inflammation in the synovium of the joint, all aforenamed modes of intraarticular treatment of chronic synovitis (together with surgical synovectomy) induce destructive degenerative changes in hyaline cartilage and in other joint structures.

The aim of the study was to select a sclerosing agent for the intraarticular treatment of chronic synovitis, able to induce fibrosis of inflamed synovium and having a minimal destructive effect upon articular cartilage and metabolism in rabbits. In a preliminary study (screening of sclerosing solutions for intraarticular treatment) the sclerosants for sclerotherapy of testicular hydrocele, epididymal, renal or hepatic cysts, of oesophageal, haemorrhoidal and leg varices (8–11) were used. Their influence on synovial inflammation and articular cartilage was unknown. The concentrations of sclerosing solutions were used as described by authors. A distinct sclerosing effect of sclerosing solutions introduced intraarticularly in inflamed synovium in chronic arthritis was found. Therefore all solutions induce more or less destructive changes in the hyaline cartilage of the joint (1). For the second stage of the study, reduced concentrations of sclerosing agents selected in the preliminary study, having a minimal destructive effect on hyaline cartilage were used for the intraarticular treatment of experimental chronic arthritis.

MATERIALS AND METHODS

The methods of induction of chronic arthritis and of evaluation of histopathological changes in synovial layer and articular cartilage were used as described in the preliminary report (1). Ten days following the last immunization active immune synovitis developed in all 30 rabbits. The rabbits were randomised into 6 groups of 5 animals each, and 1 ml of intraarticular injection of different sclerosants was introduced into the right and 1 ml of sterile physiologic saline into the left knee joint of the same rabbit (control). Each experimental group (of 5 animals) was injected with the following solutions of sclerosants: 1) Doxorubicini 1 mg; 2) Cisplastini 0.25 mg; 3) 0.5% Dioxydini; 4) 16% natrium chloride; 5) 0.05% Papaini and 6) Brulamycini 20 mg. The results were correlated to 3% Polidocanol, a sclerosant widely used in European countries for the treatment of chronic synovitis (7, 10). Because of insufficient synoviorthesis and marked destruction of cartilage, the two sclerosant solutions (96% ethanol and Cephasoline 0.25 mg) used in the preliminary study were excluded from the subsequent experimental studies. One rabbit from each group (6 animals) was sacrified 1, 2, 3, 4 and 5 weeks post sclerotherapy after 25 mg of natrium thiopental anaesthesia. Such a sequence of study permits to evaluate the dynamic of the development of histopathological signs in synovium and articular cartilage which were induced by sclerotherapy. A histopathological evaluation by grading the changes in synovium and articular hyaline cartilage of both knee joints after arthrotomy was done.

RESULTS

Grading of histopathological signs in the synovium and articular cartilage of the left knee joint of rabbits revealed distinct inflammatory changes characteristic of acute synovitis, which developed in 3 weeks and were replaced by the signs of chronic arthritis 5 weeks after immunization. In the hyaline cartilage of the left knee joint we observed formation of inflammatory pannus, as well as erosions and other structural and metabolic changes of chondrocytes, leading to atrophy of cartilage, the changes typical of destructive arthritis. All sclerosants were compared by their effect to Polidocanol which exerts a marked antiproliferative and antiinflammatory effect (joint swelling, sites of synoviocyte-A hyperplasia (by necrosis) and villous proliferation (by desquamation of vilii), foci of connective tissue disorganisation disappear and necrosis of inflammatory cells in small foci of stromal and perivascular infiltrations is induced), and stromal and vascular fibrosis and lipomatosis were found. A destructive inflammatory pannus of the cartilage turned into fibrous, and only a slight destruction of chondrocyte structure and metabolism and no atrophy of hyaline cartilage after intraarticular injection of Polidocanol were found.

A summary evaluation of fibrosis in synovium and destruction in articular cartilage upon intraarticular treatment of chronic synovitis with sclerosing solutions is presented in Table. The sclerosant solutions might be divided into two groups. The first one represents agents with deep synoviorthesis and evident destructive changes in articular cartilage without any signs of chondrocyte restoration. The action of the other one differs by the concentration of the agent introduced intraarticularly, as well as by the degree of synoviorthesis and destruction of the articular cartilage. From the first week following-intraarticular injection of Doxorubicin (2 mg/ml), in comparison to Polidocanol, the villi and synoviocyte-A necrobiosis and desquamation together with mild fibrosis and lipomatosis of the subsynovial layer of synovium remained until week 5 of the experiment. After reduced doses (1 mg/ml) of intraarticular

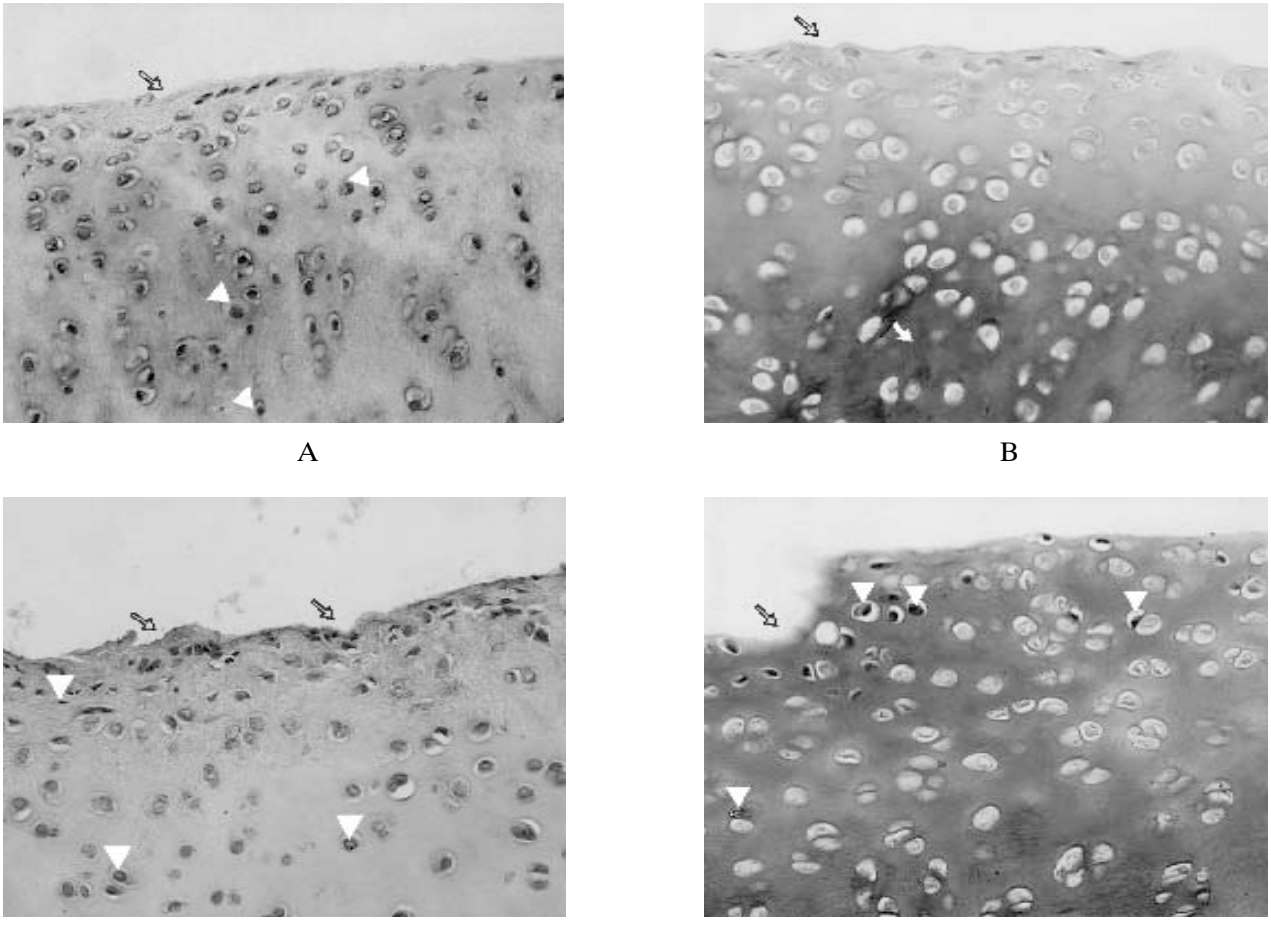
Table. **Summary evaluation of fibrosis in synovium and destruction in articular cartilage upon intraarticular treatment of chronic synovitis with sclerosing solutions**

Sclerosing solutions	Fibrosis of synovium		Destruction of articular cartilage					
			Slight		Average		Deep	
	deep	superficial	recover	unrecover	recover	unrecover	recover	unrecover
Doxorubicini	+ (+)							+ (+)
Dioxydini	+	(+)	(+)		+			
Papaini	+	(+)			+ (+)			
Cisplastini	+ (+)							+ (+)
Sol. NaCl	+	(+)			+ (+)			
Brulamycini	+							+(+)

+ – evaluation of preliminary results; (+) – evaluation of second study results.

Doxorubicini, renovation of synoviocyte-A proliferation after 5 weeks of postsclerosant injection was found. Both concentrations of Doxorubicini induced lysis of the nucleus of chondrocytes in all layers of articular cartilage, and the structure of cartilage became indistinct and composed mainly of a homogeneous proteinaceous material at the end of the experimental study. Under the influence of both concentrations of intraarticular injections of Cisplatini, the histopathological picture was similar to that of Doxorubicin. The sclerosing capacity of the agent was evident in the inflamed synovium, but the induced deep destruction of chondrocytes (picnosis of nucleus) in all zones of articular cartilage with washed proteoglycans (GAGs) to deep cartilage from the first week was not restored until 5 weeks post sclerotherapy (Fig. 1 A, B). The fibrosing capacity of Brulamycini on inflammatory synovium was evident and manifested by a necrobiosis and desquamation of proliferated villi and of synoviocytes-A layer, as well as by an inflammatory cell necrobiosis, fibrous vasculopathy and a cellular thrombus in the vascular lumen of the deep subsynovial layer. Deep usuration of the surface of cartilage, disappearance of chondrocytes having a picnotic nucleus, and an evident diminution of GAGs in all layers induced an atrophy of the cartilage with no signs of the possibility to restore (Fig. 1 C, D).

Another 3 sclerosant solutions, Dioxydini, Papaini and natrium chloride, in high concentrations induced an evident and deep fibrosis of inflamed synovium, and at reduced concentrations of the sclerosants only superficial fibrosis was found. The proliferation of villi was reduced, necrobiosis and desquamation of synoviocytes-A were evident, as well as a stromal and vascular fibrosis of synovium under the influence of high concentrations of sclerosants was found. We observed an average destruction of cartilage structure and metabolism under the influence of both concentrations of Papaini (Fig. 2 E, F) and natrium chloride (Fig. 2 C, D), while



A B C D

Fig. 1. Erosions and usuration (⇨) of the surface, irregular disarrangement, picnosis of the nucleus (▽), and GAGs in matrix of cartilage (↘) after 3 and 5 weeks of intraarticular injections of Cisplatini 0.5 mg (A) and 0.25 mg (B), Brulamycini 40 mg (C) and 20 mg (D) in rabbits with chronic synovitis. HE, Toluidine blue X 800

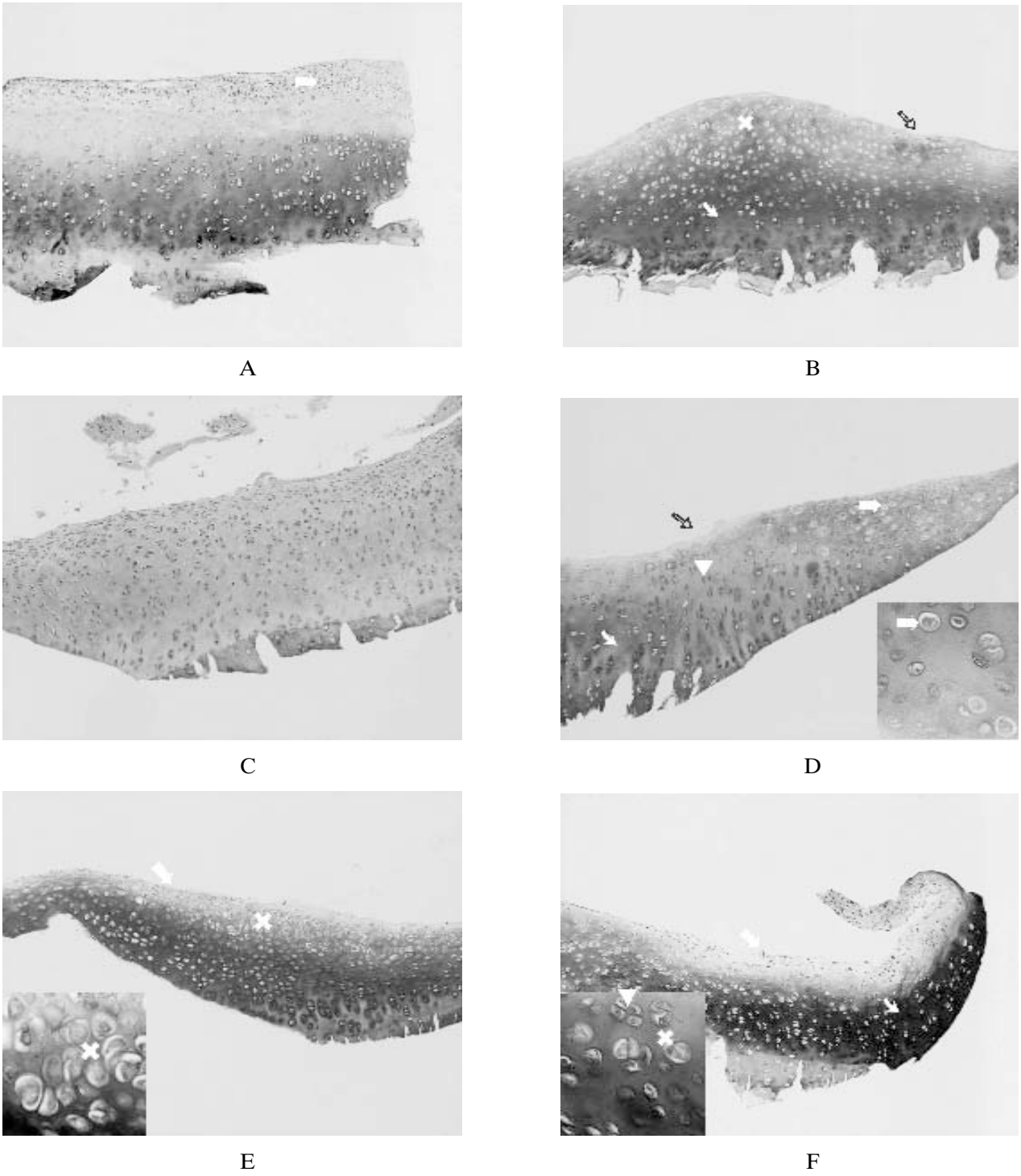


Fig. 2. Synovial plannus ( ), erosions and usuration of the surface ( ), picnosis of the nucleus and vacuolization of chondrocytes ( ), proliferated chondroblasts ( ) and GAGs ( ) in the articular cartilage of rabbits in chronic synovitis after 3 or 5 weeks of intraarticular injections of.: Dioxydini 1% (A), 0.5% (B), Sol. Natrium chloride 24% (C), 16% (D), Sol. Papaini 0.1% (E), 0.05% (F). HE, Toluidine blue. X 200, 800

only slight destruction of cartilage was found upon injection of both concentrations of Dioxydini (Fig. 2 A, B). A thicker or thinner synovial pannus and some erosions on the surface, pycnotic nucleus and cytoplasm vacuolization of disorderly situated chondrocytes in all layers of the cartilage together with diminished ability to synthesise GAGs in Papaine and natrium chloride groups was seen, but 5 weeks following sclerotherapy the autolysed chondrocytes were slightly restored and GAGs production was

increased, especially in chondrocytes of the middle and deep layers. In all sclerosants groups clusters of proliferated chondroblasts by the destroyed chondrocytes appeared, which spread into the middle zone of the cartilage from the synovial/cartilage junction.

DISCUSSION

Some sclerosants used in the study on intraarticular sclerotherapy of experimental chronic arthritis are used for clinical induction of sclerosis of testicular hydrocele, epidydimal, renal or hepatic cysts, of oesophageal, haemorrhoidal and leg varices (12–14). Their influence on synovial inflammation and articular cartilage in scientific literature is unknown. Our preliminary report screened several sclerosing solutions for the intraarticular treatment of chronic arthritis. They had a minimal destructive effect on the articular cartilage (1), and reduced concentrations of them were tested in this study. Our results showed that a high as well as reduced concentrations of Doxorubicini, Cysplatini and Brulamycini introduced intraarticularly to rabbits with chronic arthritis induced an evident fibrosis of synovium (chemical synoviorthesis), with deep destructive changes and without any tendency to restore the structure and metabolism of the articular cartilage. Such destructive action of these sclerosants mainly depends on a close contact of chemically active solutions with the surface of the articular cartilage and compels us to exclude it from an arsenal of sclerosants to be valued for intraarticular treatment of chronic arthritis.

Another group of tested sclerosant solutions Dioxydini, Papaini and natrium chloride in high concentrations induced a deep synoviorthesis with average degree of destructive changes in chondrocytes, but there remained a tendency to a slow restoration of cartilage structure (5 weeks post sclerotherapy). Reduced concentrations of these sclerosants had only a superficial and insufficient synoviorthesis capacity and some degree of destruction of chondrocytes with the remaining capacity to restore the cartilage structure by spreading the proliferated mesenchymal cells into the middle layer of cartilage from the synovium/cartilage junction. So, irrespective of the concentration of sclerosant solutions used in this study for intraarticular treatment of chronic arthritis, destructive changes of articular cartilage were revealed, but the reduced concentrations had an insufficient synoviorthesis capacity. To avoid or minimize destructive changes in the chondrocytes, different time of exposure of the articular cavity to sclerosing solutions chosen in the second study would be performed in the last stage of the experimental study.

CONCLUSIONS

1. Intraarticular injections of high concentrations of sclerosing solutions used for the intraarticular treatment in experimental chronic arthritis induced a distinct fibrosis of synovium (chemical synoviorthesis).

2. Independently of sclerosant concentration, evident destructive alterations of chondrocytes in the articular cartilage of knee joint were found. At least some of the solutions used in reduced concentrations for intraarticular treatment of chronic arthritis (Dioxydini, Papaini, natrium chloride) exhibited an ability to restore the cartilage by spreading proliferated mesenchymal cells into the middle layer from the synovial/cartilage junction.

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ANTROJI ATRANKA ĮSĄNARINIŲ SKLEROZUOJANČIŲ AGENTŲ SINOVIORTEZEI TRIUŠIŲ LĖTINIO SINOVITO EIGOJE

S a n t r a u k a

Darbo tikslas buvo ištirti sumažintų koncentracijų sklerozuojančių agentų įsąnarinį poveikį triušių lėtinio sinovito eigoje, pradinėje atrankoje (1) sukėlusių silpniausius destrukcinius sąnarinės kremzlės struktūros ir metabolizmo pokyčius. Didelės jų koncentracijos vartojamos autorių įvairiems cistiniams dariniams sklerozuoti, suleistos į sąnarį lėtinio sinovito eigoje, šalia ryškaus sinovijos dangalą fibrozuojančio poveikio sukėlė ryškią sąnarinės kremzlės destrukciją. Antroje studijoje lėtinį sinovitą sukėlėme 30-čiai triušių pagal mūsų modifikuotą Dumonde ir Glynn metodą, smulkiai aprašytą pradiniame darbe (1). Praėjus 1, 2, 3, 4 ir 5 savaitėms po sklerozantų suleidimo į dešinį kelio sąnarį, po anestezijos triušiai buvo užmušti ir sinovinio dangalo bei kremzlės pavyzdžiai iš abiejų kelio sąnarių imami histologiniam tyrimui. Tokia tyrimų seka leido įvertinti įsąnario gydymo mažesnėmis sklerozantų koncentracijomis poveikio sinovijos dangalui ir kremzlei dinamiką triušiams lėtinio sinovito eigoje. Sinovinio dangalo histopatologinis tyrimas parodė, kad sklerozuojančių agentų fibrozuojantis poveikis priklausė nuo koncentracijos: didelės sukėlė gilią subsinovinio sluoksnio fibrozę, tuo tarpu mažesnės – paviršinę fibrozę, bet abiem atvejais ryškiai sumažėjo sinoviocitų-A sluoksnio deskvamacija ir gaurelių proliferacija. Tuo tarpu mažesnės sklerozuojančių agentų koncentracijos sukėlė beveik analogiškus vidutinio stiprumo kremzlės struktūros ir metabolizmo pokyčius, kaip ir suleidus didelę koncentraciją. Vieni agentai rodė šiokius tokius kremzlės regeneracijos požymius po 4 ir 5 savaičių gydymo (Dioxydini, Papaini, NaCl tirpalas) proliferuojant mezenchiminėms ląstelėms iš sinovijos/kremzlės jungties, kiti suardė kremzlės struktūrą negrįžtamai (Cisplatini,.Brulamycini, Doxorubicini). Norėdami išvengti kremzlės destrukcijos gydant lėtinį sinovitą įsąnarinėmis sklerozantų injekcijomis, kitame šios serijos darbe bandysime sumažinti sklerozuojančio agento ekspoziją sąnario ertmėje.

Raktažodžiai: eksperimentinis artritas, sklerozuojantys agentai, sinoviortezė