Screening of Sclerosing Agents Introduced Intraarticularly for Synoviorthesis by Different Expositions in Joint Cavity in Experimental Chronic Synovitis. A Third Report

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A detailed description of histopathological changes in the synovium and hyaline cartilage induced by intraarticular injections of sclerosing solutions at high and low concentrations in rabbits with chronic arthritis has been given in the previous articles (1, 2), where also induction of chronic arthritis modified by us is described (1). In the present study we used different expositions of the joint cavity to sclerosants at proper concentrations chosen from the previous studies which induce minimal changes of hyaline cartilage or activate chondrocytes to some extent together with prominent fibrosis of the synovium in chronic arthritis of rabbits. All sclerosants were kept in the joint cavity for 5, 10, 15, 20, 25 and 30 min, and then the joiunts were punctured and washed with sterile physiologic saline. In 1 month post sclerotherapy, the synovium and hyaline cartilage of control and injected knee joints were obtained for histological examination. All expositions to sclerosants induced evident fibrosis and fibrosclerosis of the synovium. Therefore, independently of the type of sclerosant and exposition time, a destruction of hyaline cartilage was observed. Only a 25'-exposition to sol. Dioxydini 1% exhibited the ability to activate middle zone chondrocytes and to restore the cartilage structure analogously to Dioxydini 1% 5 weeks post sclerotherapy in the first experimental trail.

Key words: experimental arthritis, sclerosants, synoviorthesis, cartilage

INTRODUCTION

Chronic synovitis in patients with systemic inflammatory joint diseases leads to destructive degenerative changes of the joint. Various antiinflammatory and fibrosclerosis-inducing methods of intraarticular treatment as well as radioactive and chemical synovectomy are used to suppress chronic inflammation in the synovium effects. As mentioned in the first two articles, corticosteroids with a prolonged effect (triamcinolon, betamethasone, metipred, (3–5) are widely used to suppress inflammation in joint synovium as an intraarticular treatment. In other cases radioactive and chemical synoviortheses (1, 6, 7, 8) are used. Therefore all modes of intraarticular treatment, along with suppression of inflammation in the synovium, induce destructive changes of articular cartilage, bones and periarticular tissues. Besides, corticosteroids with prolonged activity especially when used intraarticularly, more often complicate with aseptic necrosis and chondromalation of hyaline cartilage. To avoid the side effects of intraarticular treatment for cartilage and periarticular tissues and to suppress inflammation in the synovium, substances with less drastic effects are used (9). However, in these cases the effectiveness of intraarticular treatment is short and insufficient and also contributes to joint destruction. Therefore it is reasonable to further explore more various and effective substances for intraarticular treatment of synovitis, especially for the activation of chondrocytes and restoration of the articular cartilage. Recently we have used sclerosing agents described for hepatic and kidney varicocele, leg varices sclerotherapy and for pleural

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cavity obliteration in chronic pleuritis (10–12), for intraarticular treatment at high and low concentrations in rabbits with chronic arthritis in the course of two years of experimental studies (1, 2). In previous studies we chose sclerosing solutions and their concentrations in chronic arthritis with a favourable fibrosng effect on the synovium and articular cartilage by intraarticular injections for future studies. Despite half as low concentrations fibrosis of synovium was found, but evident cartilage destruction with disarrangement and evident condensation of chondrocyte nucleis and decrease of proteoglycan synthesis were shown.

The aim of the present study was to assess the influence of different expositions to sclerosing solutions in the joint cavity on the structure and metabolism of the synovium and hyaline cartilage of knee joint in chronic arthritis of rabbits.

MATERIALS AND METHODS

The methods of chronic arthritis induction and of evaluation of histopathological changes in the synovial layer and articular cartilage were used as described in previous reports (1, 2). The rabbits were randomised into 5 groups of 6 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). All sclerosants were kept in the joint cavity for a different time (5, 10, 15, 20, 25 and 30 minutes). Then the sclerosants were punctured and the joints were washed with sterile physiologic saline.

Each experimental group was injected with the following solutions of sclerosants: Cisplastini 0.5 mg, Doxorubicini 1 mg, sol. Dioxidini 1%, sol. NaCl 24%. The results were correlated to sol. Polidocanol 3%, a sclerosant widely used in European countries for the treatment of patients with chronic synovitis.

All rabbits were sacrificed with 25 mg of sodium thiopental anaesthesia after 1 month of sclerotherapy. Such a sequence of study permits to evaluate the dynamics of the development of histopathological signs in the synovium and articular cartilage, induced by different exposition to a sclerosing agent. A histopathological evaluation by grading the changes in the synovium and articular hyaline cartilage of both knee joints after arthrotomy was done. Differences between control (left knee joint), other control group with intraarticular Polidocanol 3% and test groups (right knee joint) were statistically analysed by Student's test with $P < 0.05$ considered as significant.

RESULTS

A detailed description of histopathological changes in the synovium and hyaline cartilage of rabbits with chronic arthritis induced by intraarticular injections of sclerosing solutions at high and low concentrations have been shown in tables of previous articles (1, 2), where induction of chronic arthritis modified by us was described (1). In the present study we used different expositions of the joint cavity to sclerosants and their proper concentrations chosen from the previous studies, which induced minimal changes of hyaline cartilage or activated chondrocytes to some extent together with prominent fibrosis of the synovium in chronic arthritis of rabbits.

Sol. Cisplatini 0.5 g/ml. Disappearence of villi and synoviocytes-A lined by necrosis and desquamation, diminution of proteoglycans together with some scores of inflammation, subsynovial edema and foci of fibrosclerosis in the synovium of the right knee joint throughout all expositions to Cisplatini 0.5 g was obtained in the joint cavity as major effects. Besides, a 15' exposition of the agent in joint cavity induced lipomatosis which was seen until the end of the experiment.

Prominent erosions of the surface and usurations extending to the deep layer of hyaline cartilage from 20', 25' and 30' expositions (to the end of the experiment) to the sclerosant was seen in the joint cavity. Disarranged chondrocytes with picnosis of the nucleus, especially in the deep layer of the cartilage, and a decrease of proteoglycan synthesis were prominent from minutes 10 and 15 of exposition in the context of arthritis. Evident atrophy due to the described changes of the structure and metabolism of cartilage were also seen (Fig. 1).

Sol. Doxorubicini 1 mg/ml. All expositions to the agent in the joint cavity had a distinct anti-inflammatory effect. Necrosis and desquamation of villi, flattening and desquamation of synoviocytes-A lining in the synovium were seen from minute 15 of exposition to the agent. Slight lipomatosis that began from 5' and 10', increased on 30' of exposition and was observed to the end of the study.

A 10' and 15' exposition of the joint cavity induced numerous erosions on the surface and disappearance of surface chondrocytes on 25' and 30' of exposition. Disarranged chondrocites a with picnotic nucleus and reduced in content in the deep layer of cartilage were seen the subchondral bone where proteoglycans were washed from the top layers. Cartilage atrophy was evident (Fig. 2).

Sol. Dioxidini 1%. After 5' of exposition in the joint cavity evident proliferation of synovial villi and of synoviocytes-A to 4 and 6 layers was obtained.

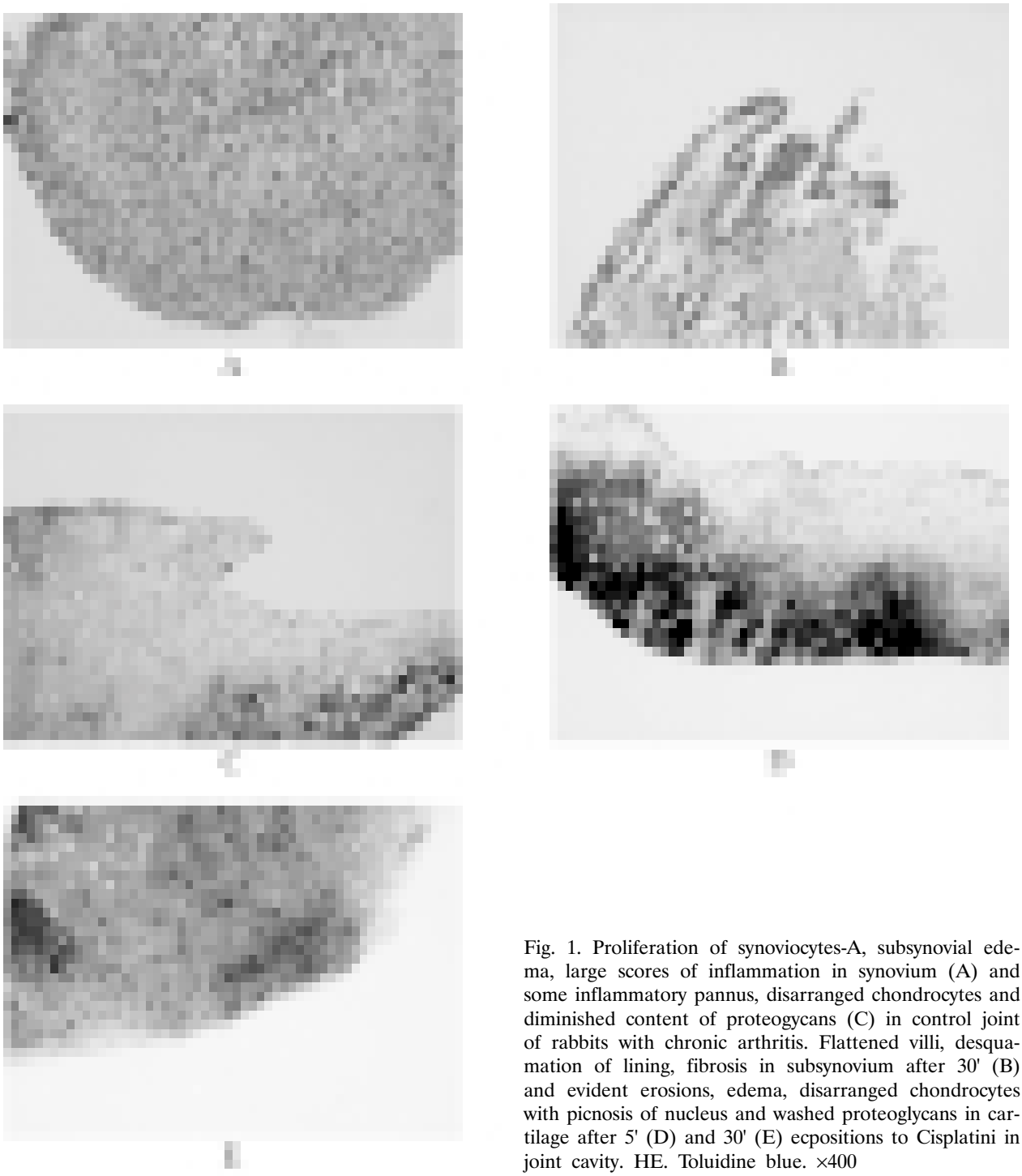

Fig. 1. Proliferation of synoviocytes-A, subsynovial edema, large scores of inflammation in synovium (A) and some inflammatory pannus, disarranged chondrocytes and diminished content of proteogycans (C) in control joint of rabbits with chronic arthritis. Flattened villi, desquamation of lining, fibrosis in subsynovium after 30' (B) and evident erosions, edema, disarranged chondrocytes with picnosis of nucleus and washed proteoglycans in cartilage after 5' (D) and 30' (E) ecpositions to Cisplatini in joint cavity. HE. Toluidine blue. ×400

Diffuse dispersion of immature plasma cells in subsynovium was seen where stasis and wall ruptures of small venules and haemorrhages were obtained on 20' of exposition. The agent activated fibroblast proliferation on 10' of exposition in the joint cavity, and a focal fibrosclerosis and lipomatosis appeared on 15'.

On minutes 10, 15 and 20 of exposition to the sclerosant a lot of erosions on the surface of hyaline cartilage were seen. Lysis of chondrocytes from 5' extended to multicellular disarrangement on 10' and its desquamation on 30' of exposition to an agent of the cartilage surface was found. Especially severely destructed were chondrocytes in the deep layer of cartilage – they had picnotic nuclei, were pushed away to small groups and decreased in number (5' and 10' of exposition). Only a 25' exposition time activated the chondrocytes of the middle zone to a

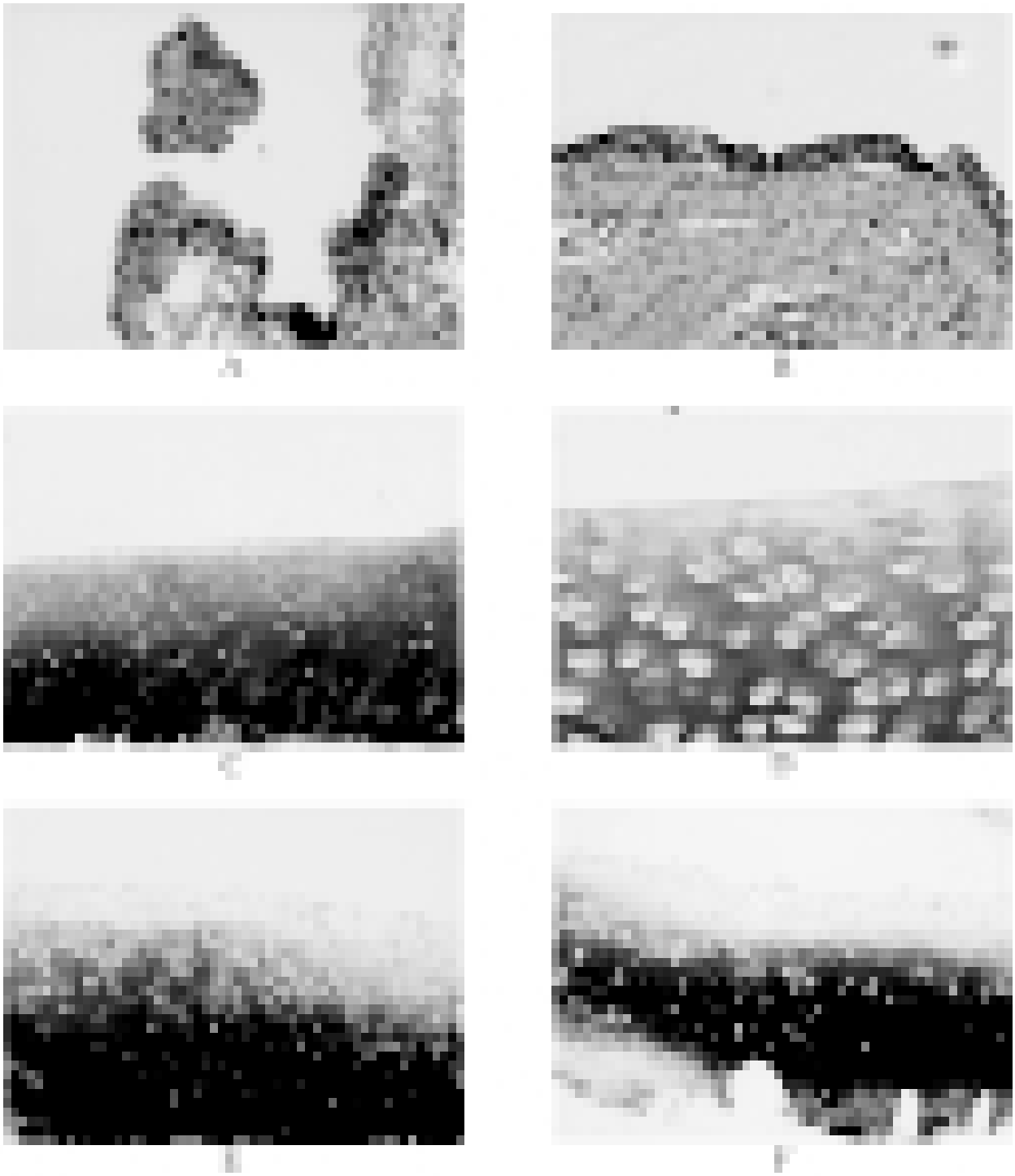

Fig. 2. Proliferation of villi, inflammation in synovium (A) and normal chondrocytes (D) with regular disarrangement of proteoglycans (C) in cartilage of control knee joint. Destruction of lining, subsynovial fibrosis, edema in synovium after 30' (B) and evident destruction of surface after 5' (E) and deep (F) chondrocytes with washed proteoglycans after 20' of exposition of Doxorubicini in joint cavity. HE. Toluidine blue. ×400, ×800

degree not observed in other expositions to the agent. Proteoglycans were held in the middle and a deep zones, but some atrophy of cartilage was seen (Fig. 3).

Sol. NaCl 24%. After a 5' exposition of the joint cavity, a flattening, infiltration with lipids and desquamation of villi were observed in the synovium. However, evident proliferation of synoviocytes-A remained to 25' and 30' of exposition to the agent. Disappearance of inflammatory cells and evident activation of fibroblast proliferation with the development of evident fibrosis, focal fibrosclerosis and fib-

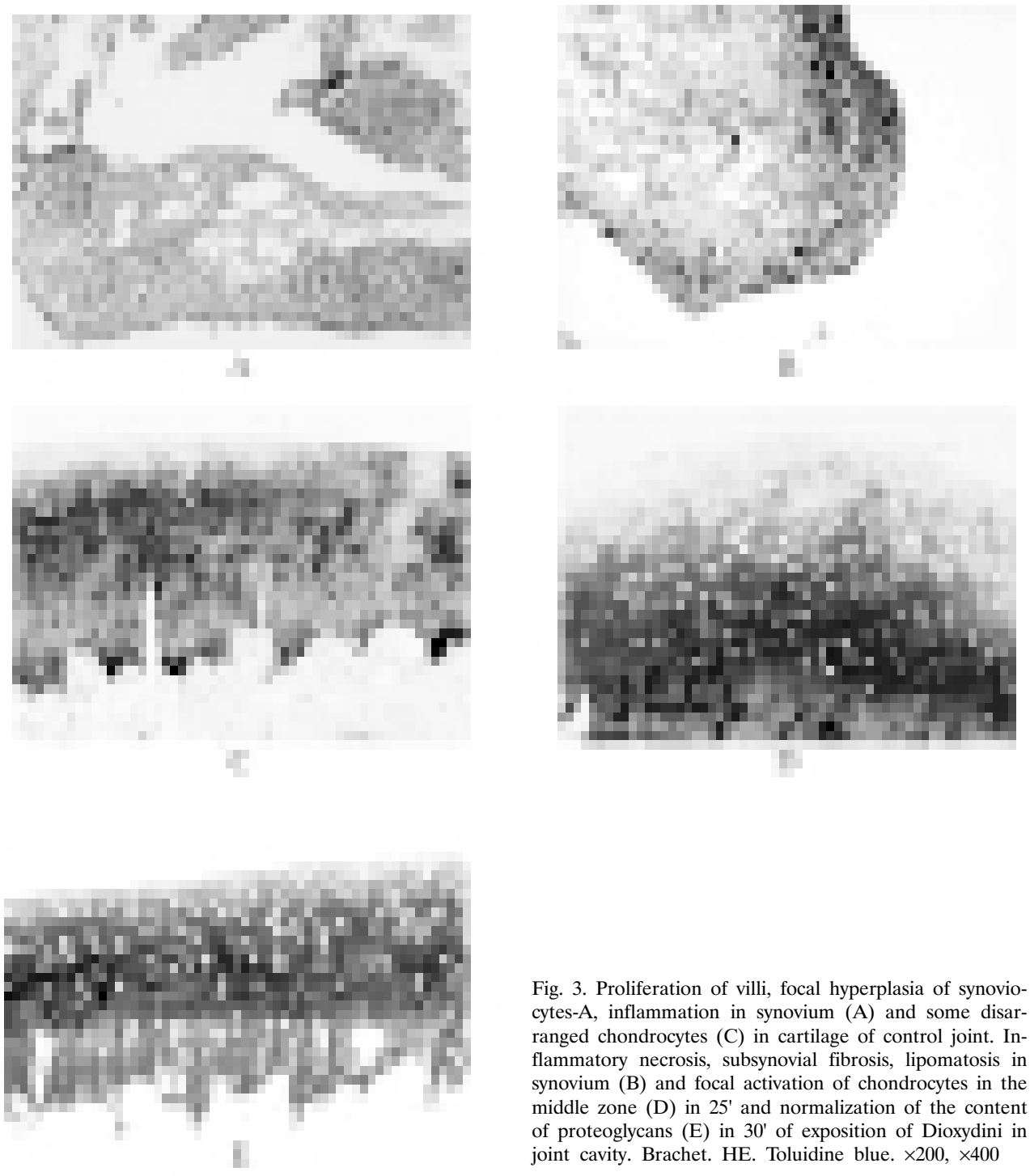

Fig. 3. Proliferation of villi, focal hyperplasia of synoviocytes-A, inflammation in synovium (A) and some disarranged chondrocytes (C) in cartilage of control joint. Inflammatory necrosis, subsynovial fibrosis, lipomatosis in synovium (B) and focal activation of chondrocytes in the middle zone (D) in 25' and normalization of the content of proteoglycans (E) in 30' of exposition of Dioxydini in joint cavity. Brachet. HE. Toluidine blue. ×200, ×400

rous angiopathy in the subsynovium began from 10' and was evident on 25' and 30' of exposition. Only a slight swelling of the synovium was seen.

The rough surface of hyaline cartilage with a lot of erosions in all expositions to the agent were seen. Homogenisation, lysis and a loss of surface chondrocytes on 30' of exposition were found. On 15', chondrocytes in the middle zone were grouped multicellularly, in the deep zone were disarranged and grouped and contained condensated chromatin and vacuoles in the cytoplasm. From the beginning of 10' of exposition, a fibrous pannus appeared on the surface, and on 25' and 30' of exposition a fibrous pannus lining the whole surface and inducing cartilage atrophy was observed (Fig. 4).

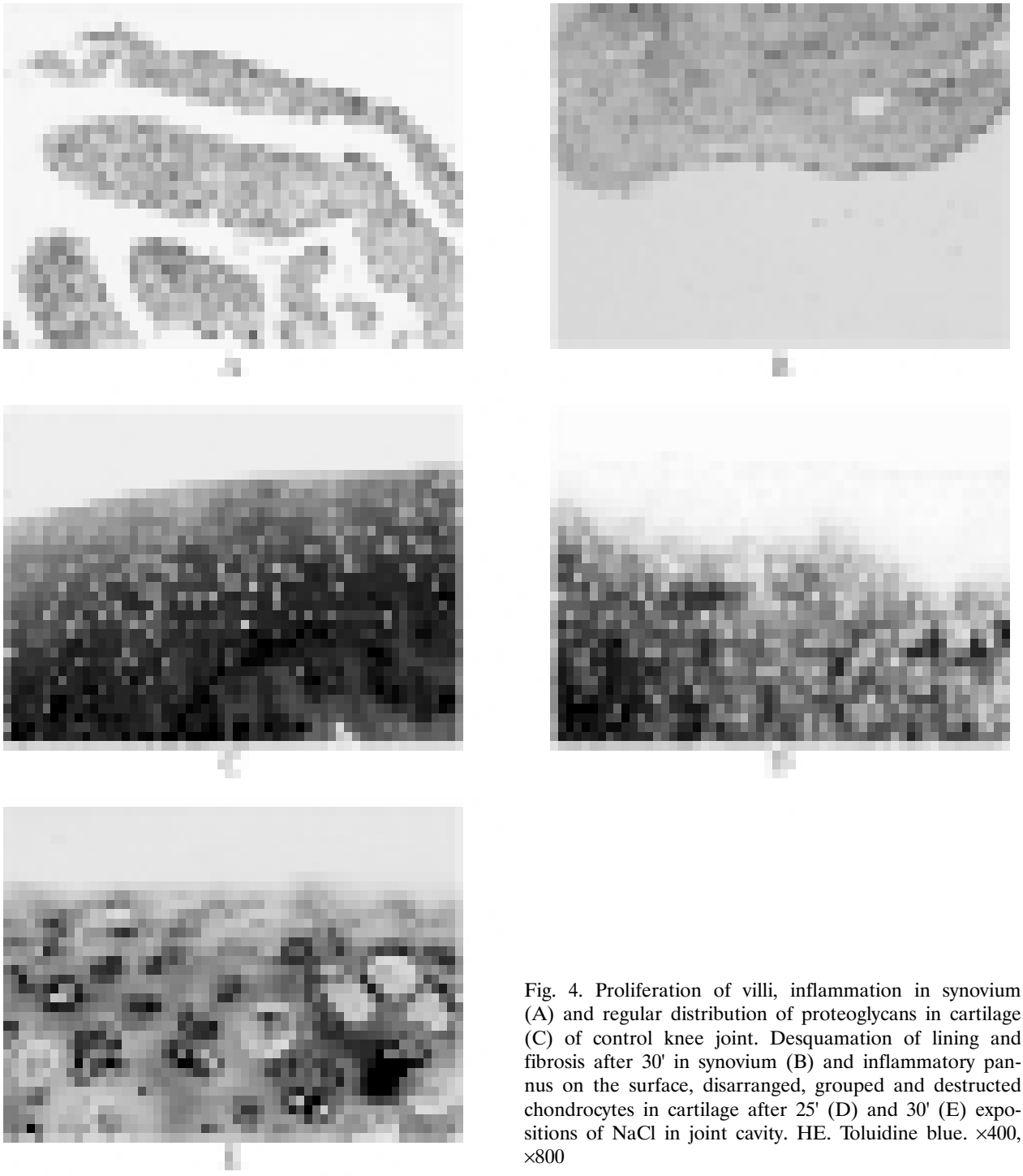

Fig. 4. Proliferation of villi, inflammation in synovium (A) and regular distribution of proteoglycans in cartilage (C) of control knee joint. Desquamation of lining and fibrosis after 30' in synovium (B) and inflammatory pannus on the surface, disarranged, grouped and destructed chondrocytes in cartilage after 25' (D) and 30' (E) expositions of NaCl in joint cavity. HE. Toluidine blue. ×400, ×800

DISCUSSION

Complex interactions between inflammatory synovium lining as antigen-presenting cells, lymphocytes, synoviocytes and their cytokines induce destruction of joint hyaline cartilage and bone and lead to joint deterioration. Therefore it is so important to suppress chronic inflammation and to avoid cartilage destruction which remains the major therapeutic target in RA. We chose the sclerosing agents and their concentrations (high and low) described for hepatic and kidney varicocele, leg varices sclerotherapy and pleural cavity obliteration in chronic pleuritis (11–13) and used for intraarticular treatment of rabbits with chronic arthritis in the previous two years of experimental studies (1, 2). The aim of the present study was to assess the influence of different expositions of the joint cavity to sclerosing solutions on

the structure and metabolism of the synovium and hyaline cartilage of knee joint in chronic arthritis of rabbits. All sclerosing agents were used at a dose of 1 ml in intraarticular injections by us for the first time. For control, joints of untreated animals and of those injected with Polidocanol 3%, a sclerosant widely used in European countries for intraarticular treatment of patients with chronic arthritis, were compared.

In the first study we used the following sclerosants: Polidocanoli 3%, Cephasolini 0.25 g/ml, Ethanol 96%, Dioxidini 1%, Doxycyclini 0.1 g/ml, Doxorubicini 5 mg/ml, Cisplatini 50 mg/ml, Papaini 0.1%, NaCl 24% and hydrogen peroxide 3%. Histological examination of the synovium and hyaline cartilage of joints revealed postinflammatory necrosis, evident subsynovial fibrosis of stroma and blood vessels and lypomatosis in the synovium induced by all sclerosing solutions used at high concentrations. A favourable synoviorthesis by caused intraarticular injections of sclerosants was accompanied by evident destruction of cartilage, beginning from erosions and usurations of the surface to disarrangement, nuclear condensation and lost number of chondrocytes in middle and deep cartilage and a decreased production of proteoglycans which induce cartilage atrophy. Therefore at the end of experiment, on week 5 following sclerotherapy by Dioxydini 1%, activation of chondrocytes in the middle zone and thickening of the focal cartilage were induced, whereas after Papaini 0.1% injections evident accumulation of chondrocytes in the middle zone by spreading of the proliferated mesenchymal cells from the synovium/cartilage junction and the entire thickening of cartilage were observed, together with an increase of proteoglycans, indicating a certain restoration of the cartilage.

For the second study, we chose sclerosing solutions inducing a lower cartilage destruction and by half diminished their concentrations: Doxorubicini 1 mg/ml, Cisplatini 25 mg/ml, NaCl 16%, Dioxydini 0.5%, Papaini 0.05%, and added a new agent, Brulamycini (20 mg/ml). Histological examination of the synovium revealed evident fibrosis induced by some solutions (Doxorubicini, Cisplatini and Brulamycini) and slighter (only focal) fibrosis caused by other agents (Dioxydini, Papaini and NaCl). However, as in the previous study with high concentrations of the agents, in the second study intense destruction of hyaline cartilage by half as low concentrations of solutions was found. Thus, it was stated that destruction of cartilage does not depend on sclerosant concentration but rather on the chemical composition of injected substances and on the structure of the tissue having no vascularization and functioning by absorbing nutrients, pathogenical and injected chemical substances from the synovial fluid of the joint cavity.

So, the third experimental study was done changing the mode of intraarticular introduction of sclerosing solutions of Cisplatini (0.5 mg/ml), Brulamycini (10 mg/ml), Dioxydini 1%, NaCl 24% and Doxorubicini (1 mg) which were maintained in the joint cavity for a different time: (5, 10, 15, 20, 25 and 30 min), and then the joints were punctured and washed with sterile physiologic saline. Histological examination of the synovium and hyaline cartilage revealed moderate fibrosis of the synovium at the end of experiment, therefore an analogous destruction of hyaline cartilage in the first two studies was independent of the exposition of the joint cavity to sclerosants. Therefore we supposed that absorbtion of sclerosant solutions by matrix proteins to the deeper zones induced swelling of the cartilage, erosions and usurations on the surface, destruction of chondrocytes by apoptosis (nuclear condensation) and decrease in chondrocyte numbers, washing of proteoglycans to the deep layers and naked collagen fibres – the main structural and metabolic changes of destruction and atrophy of cartilage similar to those of osteoarthritis in patients (13). Only a 20' exposition to sol. Dioxydini 1% caused focal activation of chondrocytes in the middle zone of cartilage analogously to that of 5 weeks post sclerotherapy in the first study. Attention should be paid to sol. Papaini 0.1%, which activates mesenchymal cells from the synovium/cartilage junction to spread into the middle zone of cartilage and to restore the structure and metabolism of cartilage as we have shown in the first experimental study.

CONCLUSIONS

All expositions to sclerosing solutions in the joint cavity, used for intraarticular treatment of chronic arthritis, induced evident supression of inflammation, fibrosis and fibrosclerosis of subsynovial stroma and blood vessel wall of the synovium. Therefore, independently of the type of sclerosant and exposition time in the joint cavity a destruction of hyaline cartilage was observed too.

Only a 25' exposition of the joint cavity to sol. Dioxydini 1% used for intraarticular injection in chronic arthritis of rabbits activated the chondrocytes in the middle zone and focally restored the cartilage structure, analogously to the same concentration of Dioxydini five weeks post sclerotherapy in the first experimental study.

Received 20 June 2002
Accepted 10 September 2002

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ĮSĄNARINIO GYDYMO SKLEROZUOJANČIŲ AGENTŲ KONTROLĖ PAGAL EKSPOZICIJOS LAIKĄ SĄNARIO ERTMĖJE ESANT TRIUŠIŲ LĖTINIAM ARTRITUI. TREČIOJI STUDIJA

S a n t r a u k a

Straipsnyje pateikiami baigiamojo eksperimento duomenys, gauti priklausomai nuo sklerozanto ekspozicijos trukmės sąnario ertmėje, bei aptariami visų trijų tyrimų gauti duomenys. (Pirmųjų duomenys aprašyti *Acta medica Lituanica* 2000.7: 166–170; 2001.8: 193–197 1,2.) Trečiajam bandymui buvo atrinkti vaistai, sukėlę mažiausius pokyčius hialininėje kremzlėje, o bandymo pabaigoje – kremzlės atsistatymo požymius. Šie vaistai sąnaryje buvo išlaikomi 5, 10, 15, 20, 25 ir 30 minučių, po to išpunktuojami, o sąnarys praplaunamas fiziologiniu skiediniu. Kontrolinio ir bandomojo kelio sąnario sinovija ir kremzlė ištirta histologiškai.

Paaiškėjo, kad sinovinio dangalo fibrozę sukėlė visi naudoti sklerozantai, nepaisant vaisto ekspozicijos trukmės sąnario ertmėje, tačiau visi vaistai pakenkė sąnario hialininei kremzlei sukeldami ryškią jos destrukciją. Eksperimento pabaigoje 1% Dioksidino 20' ekspozija sąnario ertmėje lėmė chondrocitų aktyvumą vidurinėje zonoje ir židininį kremzlės atsistatymą, tolygų tos pačios koncentracijos dioksidino poveikiui į kremzlę po 5 savaičių (pirmo tyrimo duomenys).

Raktažodžiai: eksperimentinis artritas, sinoviortezė, sklerozantai, kremzlė