CHEMICAL SYNOVECTOMY OF THE KNEE IN RABBITS – OXYTETRACYCLINE VERSUS AETOXYSKLEROL – A EXPERIMENTAL STUDY

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Abstract

*Introduction.*Recurrent haemarthroses will inevitably lead to significant hypertrophic synovitis in patients with haemophilia (PwH), progressive joint cartilage degradation, ultimately resulting in haemophilicarthropathy with significant functional impairment of the affected joints. The degree of haemophilicsynovitis is directly related to anincrease in bleeding frequency in the affected joint.Synoviorthesis or non-surgical synovectomy is aa therapeutic method which consists in injection of a substance in to the joint, which acts on the synovial membrane by means of a fibrosis that constricts the subsynovial plexus and thus prevents future bleeding. There are two groups of preparations: chemical and radioactive isotopes. *Material and methods.*Twenty two albino New Zeeland White specific pathogen free rabbits were used for the study. From one of them synovial membrane was harvested from both knees and send for anatomopathological examination. The remaining rabbits were injected into both knees once a week for 4 weeks with autologous blood (2 ml) harvested from the safenous vein, mimicking the pattern of repeated hemarthroses that the patients with haemophilia experience. After this synovial membrane and articular cartilage were harvested from both knees from a second rabbit and send for anatomopathological examination to observe intraarticular damage. The remaining rabbits were divided in two groups and oxytetracycline (200 mg/ml) and aetoxysklerol 1% (20 mg/ml) were injected into their left knee, while the right knee was injected with saline solution in each group of rabbits once a week for a period of 4 weeks. After that all rabbits were euthanized and synovial membrane and joint cartilage were harvested. Theanatomopathological specimens were stained with hematoxylin-eosin and examined under optic microscopy. *Results and discussions.* No problems related to the procedures were encountered, except for discrete ambulatory problems after injection of blood into the knee joints. The general status of all rabbits was good during the whole period of the experiment. Repeated hemarthroses into the knee resulted in proliferation of the synoviocits with inflammatory signs resembling acute synovitis and higher magnification levels in optical microscopy revealed presence of hemosiderin and inflammatory cells. This proliferation of the synovium and neovascularization of the subsynovial layer results in an inflamed, villous, friable and highly vascular synovial tissue.The specimens from the rabbits injected with oxytetracycline and aetoxysklerol showed fibrosis, regeneration of the synovial tissue and controlled reparation, with slide enlargement of synovial tissue, less irrigated, and less prone to rebleed in 100% of cases. *Conclusions.* Synoviorthesis should be the first choice of treatment for persistent synovitis of the joints in patients with haemophilia. It is a simple procedure, which eliminates the risks associated with surgery and is also cost-efficient. Preliminary experimental data show a good efficiency of both oxytetracycline and aetoxysklerol as materials used for chemical synovectomy in rabbits with acute synovitis of the knees. There is still need for further experimental data gathering and dosage adjusting before optimal use of these substances in the treatment of haemophiliacs.

Key words: hemophilia, rabbit, chronic synovitis, synoviorthesis, aetoxysklerol,, oxytetracycline

Introduction

Recurrent haemarthroses will inevitably lead to significant hypertrophic synovitis in patients with haemophilia (PwH) , progressive joint cartilage degradation, ultimately resulting in haemophilicarthropathy with significant functional impairment of the affected joints. The degree of haemophilicsynovitis is directly related to anincrease in bleeding frequency in the affected joint(1). There are two basic types of procedures for synovial control: medical synovectomy (or synoviorthesis) and surgical synovectomy (open or arthroscopic.) It is commonly accepted today that synoviorthesis is the procedure of choice, and that surgical synovectomy should be performed only if a number of consecutive synoviortheses fail to stop or diminish the frequency of recurrent haemarthrosis(2,3).

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A synoviorthesis consists of the intra-articular injection of a certain material with the aim of `stabilizing' (orthesis) the synovial membrane of a joint (synoviorthesis) by means of a fibrosis that constricts the subsynovial plexus and thus prevents future bleeding. There are two groups of preparations: chemical and radioactive isotopes. Thus, the main indication for a synoviorthesis in a haemophilic joint is hypertrophic synovitis and recurrent bleeding. Synoviorthesis has been utilized for more than 25 years(4-7).

Aim of the present study

The aim of the present study is to test the efficiency of new chemical substances for synoviorthesis and introduce them to clinical practice. Oxytetracycline has been used on an experimental basis before (8-11),aetoxysklerol was choosen because of very good fibrotic properties in the treatment of pediatric hemangiomas (personal experience). The rabbit was chosen for this experiment because of the histological similarity of the animalssynovium with the human one (12-18).

Material and methods

Twenty two albino New Zeeland White specific pathogen free rabbits were used for the study. All surgical interventions were conducted under general anesthesia with ketamine associated with xylazine. The rabbits were prepared for surgery and all rules of asepsia and antisepsia were respected. From one of the rabbits synovial membrane was harvested from both knees and send for anatomopathological examination (Fig.1-3). The remaining rabbits were injected into both knees once a week for 4 weeks with autologous blood (2 ml) harvested from the safenous vein, mimicking the pattern of repeated hemarthroses that the patients with haemophilia experience . After this synovial membrane and articular cartilage were harvested from both knees from a second rabbit and send for anatomopathological examination to observe intraarticular damage.Intraoperative findings were : amber color,brown staining (hemosiderin) and thickening of the synovium at the level of both knees, the presence of numerous envelopes, secondary hyperaemia, increased vascularization of the synovium with subsequent tendency towards bleeding(Fig. 4) .The synovium appeared thickened, inflamed and hypervascular(Fig.5, 6).These findings together with the anatomopathological ones correspond to the ones observed in patients with haemophilia after repeated intraarticular bleedings and in experiments done by other authors (8-11, 19-25).

The remaining rabbits were divided in two groups and oxytetracycline(50 mg / kg body weight) and aetoxysklerol 1% (1 mg / kg body weight) were injected into their left knee, while the right knee was injected with saline solution in each group of rabbits (1 ml), once a week, for a period of 4 weeks. After that all rabbits were euthanized by injection of Euthanyl (100 mg / kg body weight) and synovial membrane and articular cartilage were harvested in 30 minutes after death. Macroscopic intraoperative findings were a shrinking of the synovium in the joints injected with aetoxysklerol and oxytetracicline and fibrosing aspect in all rabbits with reduction of vascularization (Fig . 7).The anatomopathological specimens were stained hematoxylin-eosin, Perls, Tricrome Gömöri, Tricrome Masson and examined under optic microscopy (Fig. 8-11).

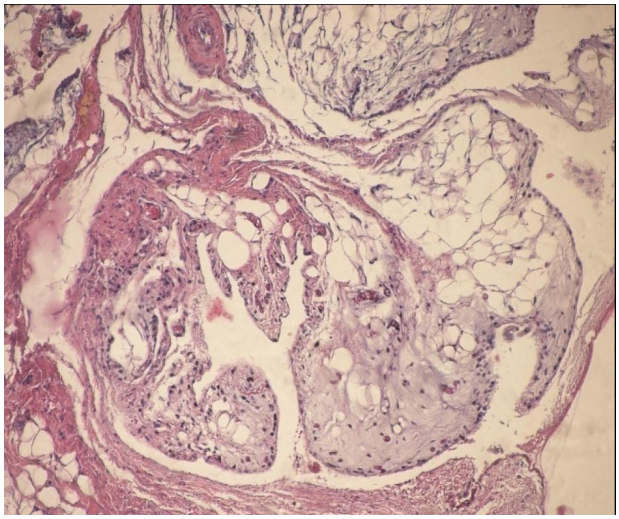
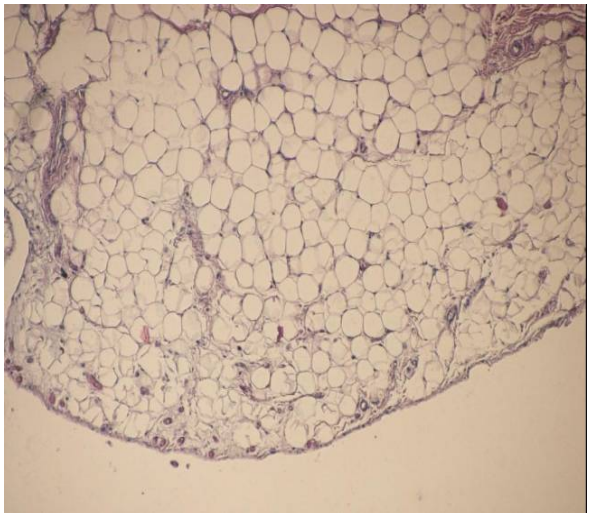


Fig. 1 Hematoxylin-eosin staining, magnification100X: Intact synovial tissue, loose connective tissue with capillary-type vessels in small numbers.



Hematoxylin-eosin staining, magnification 200 X: free synovium, synoviocytes located on a single row, reduced subjacent loose connective tissue and fatty tissue well represented.

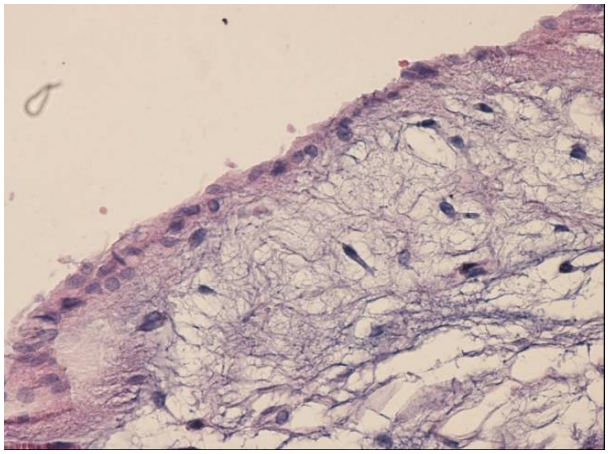


Fig. 3 Hematoxylin-eosin staining, magnification400X: loose connective tissue richin fundametal substance, small capillary-type vessels.

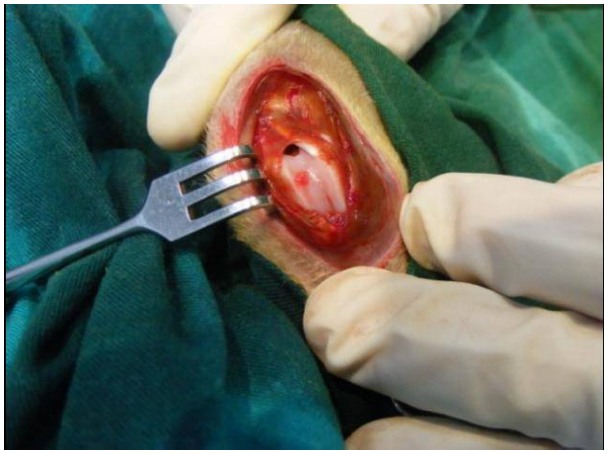


Fig. 4. Intraoperative aspect of the synovium after repeated injections of autologous blood.

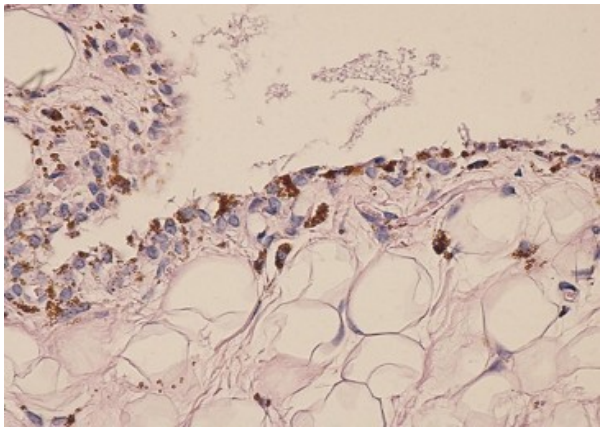


Fig. 5 Hematoxylin-eosin stain, magnification 400X : Proliferated synoviocytes across layers associated with hemosiderin loaded hystiomacrofages.

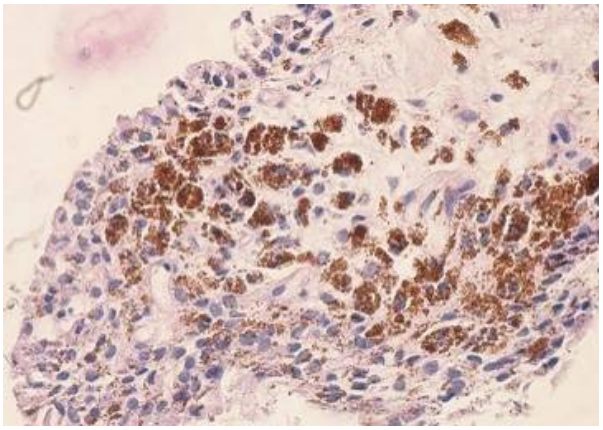


Fig. 6 Hematoxylin-eosin stain, magnification400X: Intensely proliferated synoviocytes, subjacent hystiomacrofages loaded with hemosiderin.

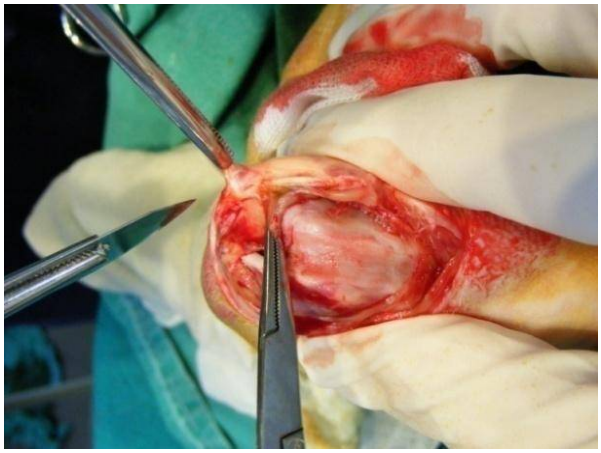


Fig. 7. Macroscopic aspect of the joint injected with aetoxysklerol.

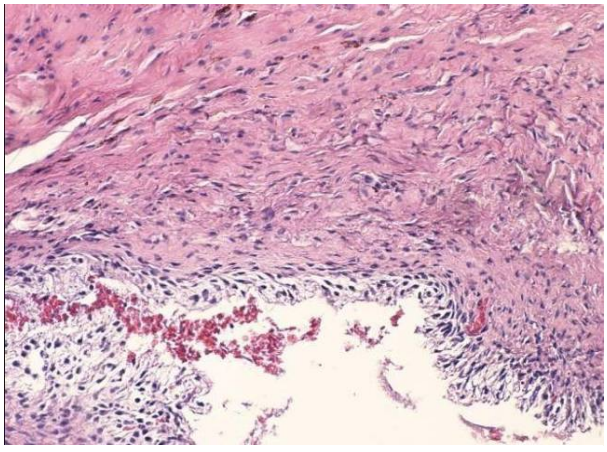
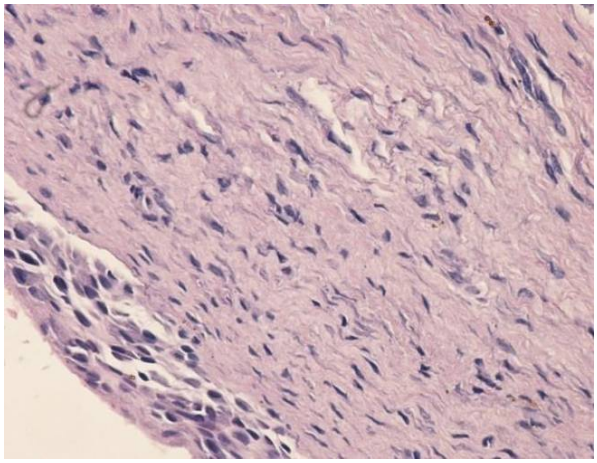


Fig. 8.Hematoxylin-eosin stain, magnification 200X: Proliferation of synoviocites, marked fibrosys and reduction of vessels.



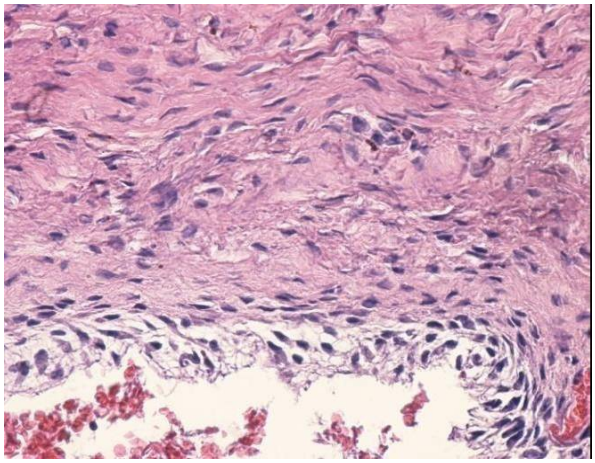


Fig. 9., Fig.10., Hematoxylin-eosin stain, magnification400X (detail): Proliferation of synoviocites, marked fibrosys and reduction of vessels (slide enlargement of synovium with appearence of tissue less irrigated and thus less prone to rebleed).

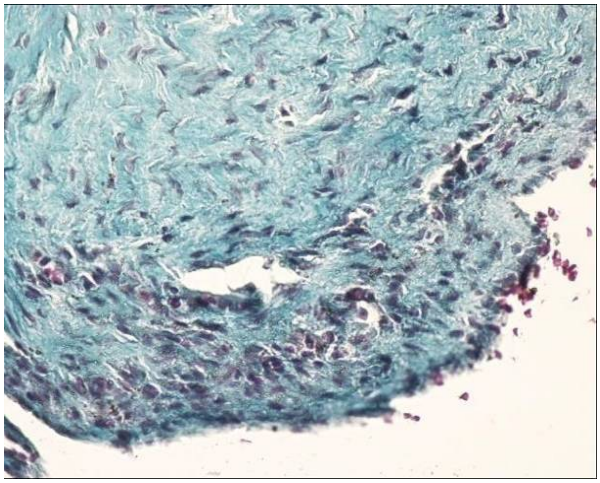


Fig. 11.Tricrome Masson stain , magnification 200 X: Marked fibrosis of the synovium.

Results and discussions

Repeated hemarthroses into the knee resulted in proliferation of the synoviocits with inflammatory signs resembling acute synovitis and higher magnification levels in optical microscopy revealed presence of hemosiderin and inflammatory cells. This proliferation of the synovium and neovascularization of the subsynovial layer results in an inflamed, villous, friable and highly vascular synovial tissue.

The specimens from the rabbits injected with oxytetracycline and aetoxysklerol,examined under optic microscopy, showed fibrosis, regeneration of the synovial tissue and controlled reparation, with slide enlargement of synovial tissue, less irrigated, and less prone to rebleed in 100% of cases (Fig. 8-11). This demonstrates the efficiency of chemical synoviorthesis with these two substances in the animal subject.Aetoxysklerol appeared to be more efficient than oxytetracycline, with a more pronounced fibrosis in the examined samples.

There were absolutely no local complications in the studied lot of rabbits and as a systemic complication we noted only pain which responded well to analgesic treatment over a short period of time (usually 24-48 hours). The general status of all rabbits was good during the whole period of the experiment.

Other autors have used osmic acid, rifampicine and oxytetracycline for chemical synovectomies in the treatment of patients with hemophilia.

The most commonly used chemicals have been osmic acid and rifampicin. In fact, they have been utilized as an alternative to radioactive agents because of lack of availability or fear of radiation as a potential source of malignancy.

In 1973, Menkes et al. (19) reported their experience with the use of intra-articular osmic acid. Their results were

mixed and this procedure never achieved wide popularity. Caruso, in the 1980's was one of the first to use rifampicin as a chemical agent for the treatment of synovitis associated with rheumatoid arthritis (20). Rifampicin waschosen for its proteolytic and fibrinolytic properties. Despite encouraging early results there was, however, a high failure rate.

Salis et al.(21) retrospectively reviewed their experience with non-surgical synovectomy in the treatment of recurrent haemarthrosis with arthropathy in patients with von Willebrand's disease, which is the most common inherited bleeding disorder, with an overall prevalence in the general population of 0.8-1.3%. Haemarthrosis occurs mainly in the most severe forms of the disease (type 3), with a frequency of 3,5-11%, and can cause severe arthropathy similar to that seen in haemophilia. Four of six patients had type 3 disease and the remaining two had type 2 disease. The age range was 13-63 years. The frequency of haemarthrosis prior to synovectomy was 1-4 per month. One (n = 2) or both (n = 1) knees were treated in four cases, one (n = 1) or both (n = 1) ankles in three cases and an elbow in one case. ^{90}Y was used in a dose of 5millicuries (mCi) (or185 mega becquerels (MBq)) for one knee, ^{186}Re in a dose of 2 mCi (or 74 MBq) for two ankles and the elbow andosmic acid for two knees and one ankle. Clinical and radiological results were evaluated 6 months aftersynovectomy using the World Federation of Haemophilia score. Radiological lesions remained stable and clinicalmanifestations improved in every case ($P < 0.05$). Five patients achieved a complete remission. Safety was satisfactory and there were no complications. The clinical efficacy of synovectomy, using radiocolloids or osmic acid in arthropathy caused by von Willebrand's disease, seems similar to that in haemophilia.

Caviglia et al.(22,23) reported that, for many years, rifampicin has been used empirically for the treatment of chronic haemophilicsynovitis with encouraging results. A clinical study was performed on 48 haemophilic patients (48 joints). Seventeen elbows, eight knees and 23 ankles were treated. The mean age of the patients was 6 years (range 4-23 years) and the mean follow-up was 29 months (range 24-53 months). Overall, 40 excellent and eight good results were obtained. The average number of weekly injections of rifampicin was 3.06 (range 1-10 injections). Eight patients experienced pain on the first injection, which subsided gradually with the subsequent procedures.

Synoviorthesis with rifampicin seems to be a good method for the treatment of haemophilicsynovitis, especially insmall joints (elbows and ankles) and in younger children.

Fernandez-Palazzi et al. (24) also assessed the effectiveness of intra-articular rifampicin in haemophilic patients. Two hundred and fifty milligrams of rifampicin was injected into the elbow and ankle joints and 500 mg was injected into knee joints with 3-10 ml of lidocaine, depending on the joint size. The injections were repeated once a week for 7 weeks.This paper reports on the results of 38 patients with 39 joints with more than 3 years followup (mean 1.8 years). There were 22 knees, nine elbows and eight ankles. Subjectively, there were excellent results in 21 joints (11 knees, six elbows and four ankles), good results in 15 joints (eight knees, three elbows and four ankles), fair results in two knees and a poor result in one knee. Objectively, results obtained were excellent in 20 joints (11 knees, six elbows and three ankles); good in 17 (nine knees, three elbows and five ankles); fair in one knee and poor in one knee.

Radossi et al.(25) have used intra-articular injections of rifamycin.Among a large cohort of nearly 500 patients, they treated 28 patients during a 2-year period. The patients followed an on-demand replacement therapy programme and developed single or multiple joint chronic synovitis. The indications for synoviorthesis were symptoms of chronic synovitis referred by patients reported in a questionnaire.

In Radossi's series there were five patients with inhibitors to factor VIII. Their average age was 34 years. Rifamycin (250 mg) was diluted in 10 ml of saline solution and 1–5 ml was then injected into the joint. The follow-up ranged from 6 to 24 months. Thirty-five joints were treated with 169 infiltrations in total. Rifamycin was injected once a week for 5 weeks, that is the patient had to come to hospital at weekly intervals. Twenty-four procedures were considered effective in 19 patients according to the evaluation scale, while six treatments were considered fair to poor. Five patients (six joints) with anti-factor VIII inhibitors were treated. In four joints the results were good, while in the two remainingjoints the results were poor.

Oxytetracyclineis a broad-spectrum antibiotic, active in both Gram-positiveand Gram-negative bacteria, especially filarias andrickettsias. In intravenous injections it was noted that phlebitisdeveloped because of it'sirritative property. This antibiotic waswithdrawn from human use as a result of this irritating actionwhen injected intravenously, and is now only used for veterinarianpurposes. This irritating action is what we were searching for, in order to produce fibrosis of the synovial membrane.

Oxytetracycline has been used before on a experimental basis and later during clinical trials by Fernandez-Palazzi et al.(8-11). The autors had excellent results in 32 joints, good in 16 joints, fair in two joints and poor in four (two underwent surgical intervention). In spite of these being early results, they were very satisfactory in the opinion of these autors, especially in relation to pain, diminution of joint diameter and increase of ROM. Patient satisfaction, despite some failures, was above 90%.

Aetoxysklerol has never been used for chemical synovectomy but was choosen because of very good fibrotic properties in the treatment of pediatric hemangiomas and lymphangiomas (personal experience).

Synoviorthesis can be performed at any age in haemophilia patients. Performing an intra-articular injection in a very young child does pose the problem of patient cooperation which may require conscious sedation or even generalanaesthesia. It is possible to perform multiple synoviorthesis in a single session. The other non-invasive alternative to chemical synovectomy would be radiosynovectomy.

There is always a cocern about the use of radiosynovectomy, the effects on joint cartilage and the incidence of cancer after this kind of treatment. Jahangier et

al.(26) have found that radiation synovectomy with ^{90}Y for persistingarthtritis has harmful effects in vitro on human cartilage that cannot be prevented by co-administration of glucocorticoids.These results urge for a more detailed in vivo evaluation of cartilage changes afterradiosynovectomy. Dunn et al.(27) reported about two patients which developed acute lymphocytic leukemia(ALL), one T-cell ALL and one precursor B-cell ALL, within one year of radioactive synovectomy with ^{32}P. There are also new radiocolloids tested. Calegaro et al.(28) reported of the results in the treatment of chronic haemophilicarthropathy with 153-samarium hydroxyapatite(^{153}Sm-HA) in 31 patients with haemophilia.

Conclusions

Synoviorthesis should be the first choice of treatment for persistent synovitis of the joints in patients with haemophilia. It is a simple procedure, which eliminates the risks associated with surgery and is also cost-efficient.

Synoviorthesis is a highly effective procedure that decreases both the frequency and the intensity of recurrent intra-articular bleeds related to joint synovitis. The procedure should be performed as soon as possible to minimize the degree of articular cartilage damage, which, based on many studies, is irreversible. It can also be used in patients with inhibitors with minimal risk of complications. On average, synoviorthesis has a 75-80% satisfactory outcome in the long term. From the clinical standpoint, such efficacy can be measured by the decrease in the number of haemarthroses, with complete cessation for several years in some cases. Synoviorthesis of any kind is a highly cost-effective method compared to open or arthroscopic synovectomy. One should bear in mind that in 20-25% of cases, synoviorthesis fails to control haemarthroses. In such cases, it can be repeated.

Based on the animal experiment we propose the introduction of chemical synovectomy with oxytetracycline and aetoxysklerol in clinical practices(29). For oxytetracycline we propose injections depending on the affected joint:

- for knees: 250 mg associated with 5 ml anaesthetic
- for elbows: 100 mg associated with 2 ml anaesthetic
- for ankles : 50 mg associated with 1 ml anaesthetic

The dosis may vary depending on the age and body weight of the patient. In small aged patients we also recommend the use of general anesthesia.

The maximum daily dosis of aetoxysklerol we recommend is 2 mg/kg body weight. Due to the fact that aetoxysklerol is also a local anaesthetic we do not recommend the associated use of lidocaine, to prevent secondary reactions. A initial quantity of 1-2 ml of aetoxysklerol 2% can be injected at the level of small joints and 3-4 ml of aetoxysklerol 2% can be injected at the level of large joints. The dosis can be increased to the maximum daily dosis if no secondary reactions appear. Multiple joints can be treated in the same therapeutic session.

Preliminary experimental data show a good efficiency of both oxytetracycline and aetoxysklerol as materials used for chemical synovectomy in rabbits with synovitis of the knees. There are also other advantages of this therapeutic method such as low cost, easy technique, immediate therapeutic effects, short period of treatment and low consumption level of coagulation factor. There is still need for further experimental data gathering and dosage adjusting before optimal use of these substances in the treatment of patients with haemophilia.

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