Knee Surg Sports Traumatol Arthrosc (2011) 19:94–98
DOI 10.1007/s00167-010-1154-9

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Adhesion reduction after knee surgery in a rat model by Mitomycin C

Baris Kocaoglu · Umut Akgun · Ufuk Nalbantoglu · Oguz Poyanlı · Mustafa Karahan

Received: 30 December 2009 / Accepted: 9 April 2010 / Published online: 8 May 2010

Abstract The purpose of this study was to investigate the effect of Mitomycin C on reduction in intra-articular fibrotic adhesion formation after knee surgery. Twenty female Wistar Albino rats were divided into two equal study groups. After skin incision, the right knees of animals were opened through a medial parapatellar approach, and the medial and lateral sides of the femoral condyle were exposed. A partial capsulotomy and synovectomy were performed. In group I, Mitomycin C was applied in the articular cavity of rats after hemostasis. An identical volume of sterile normal saline was applied in group II, which served as controls. All rats received weekly (6 weeks) intra-articular both Mitomycin C and saline starting from the day of operation. Animals were killed at 45 days after surgery. The presence and severity of osteo-capsular adhesion was assessed with both macroscopic and microscopic adhesion scoring systems. Results of macroscopic observations showed a significant reduction in both incidence and severity of adhesions in group I compared to group II. Histologically, adhesions in group I were thinner with less collagenic fibers. Microscopic determination of adhesion formation was less in group I, and the difference between groups was statistically significant. Our results suggest that post-operative administration of Mitomycin C may be considered a promising preventive therapy for the reduction in fibro-adhesive scars and consequent stiffness after knee surgery.

Keywords Knee surgery · Adhesion formation · Mitomycin C · Prevention

B. Kocaoglu (✉) · U. Akgun · U. Nalbantoglu
Department of Orthopaedics and Traumatology,
Acibadem University Faculty of Medicine, Barbaros mah,
Kentplus 1, Etap, C1 blok D:52, Yenisahra, Istanbul, Turkey
e-mail: bariskocaoglu@gmail.com

O. Poyanlı
Department of Orthopaedics and Traumatology,
SSK Goztepe Hospital, Istanbul, Turkey

M. Karahan
Department of Orthopaedics and Traumatology,
Marmara University Faculty of Medicine, Ciftehavuzlar,
cemil topuzlu cad. No:37, daire:2, Kadikoy, Istanbul, Turkey

Introduction

Arthrofibrosis of the knee represents an important concern after knee surgery or trauma. The formation of restrictive adhesions after knee surgery can alter knee biomechanics, leading to stiffness and other severe functional impairments with subsequent pain and cartilage degeneration [1, 2, 5]. The physiological mechanisms of adhesion formation still remain unclear; however, several authors have described the mechanism as an abnormal wound healing process with the involvement of cellular and biochemical components such as cytokine and growth factors leading to fibrous tissue hyperplasia [2–4, 7, 13].

The ideal solution for adhesion seems to be the application of an anti-adhesion device to prevent formation of scar tissue. Various methods have been attempted such as continuous corticosteroid administration, intra-articular chitosan injection and local administration of FGF-2 antibodies and a hyaluronan derivative gel [2, 9, 10, 13]. However, the results from human clinical trials have been conflicting or involved adverse events [11].

Numerous substances have been used to prevent fibrosis formation in the knee joint with varying effectiveness, but reports on Mitomycin C (MMC) inspired us to investigate its role in prevention of fibrosis. MMC is produced by Streptomyces cuspidatus and initially used as an aminoglycoside antibiotic, and it is an anti-neoplastic agent that has been used safely in humans since 1983 [3, 8, 15]. Its mechanism of action is through cross-linking of DNA. Its proposed mechanism of action is through prevention of fibroblast proliferation, a major step in scar formation, by blocking DNA synthesis by cross-linking DNA [3, 8]. In animal models, MMC has been found to be effective in preventing post-operative formation of intra-peritoneal adhesions by inhibiting fibroblastic proliferation. MMC is active against all cell lines and safely has been used topically in humans. It has been used successfully in ophthalmologic surgical procedures and in otorhinolaryngology [3, 8].

The purpose of this study was to investigate the effect of MMC on fibrotic adhesion reduction after knee surgery. Our results suggest that post-operative surgical administration of MMC may be considered a promising preventive therapy for reduction in fibro-adhesive scars and consequent stiffness of the knee.

Materials and methods

Animal model

Twenty female Wistar Albino rats weighting between 350 and 450 g were used for this study. Permission was given to use of laboratory animals by Research Etiquette Commission for animal experiments. Animal care complied with the guidelines of the authors' institution and the National Institutes of Health and national law on the care and use of laboratory animals.

The animals were given standard chow and water ad libitum. This animal model has been chosen as commonly used for general surgery studies; specifically, it is widely accepted as preclinical model for adhesion prevention products' evaluation [2]. Twenty animals is the minimal number to obtain reliable results and detect significant differences among the two groups. Before surgery, animals were randomly assigned to one of two groups of 10 animals each (group I, treated; group 11, control).

The following surgical procedure was followed in order to induce a high incidence of adhesions. Anesthesia was performed with an intraperitoneal injection of pentothal (50 mg/kg). A preoperative dose of intramuscular cefazolin sodium (0.1 mg/kg) was administered for infection prophylaxis. In aseptic conditions, the right knee joint was shaved with an electric clipper, prepped with iodine solution and then aseptically draped. After skin incision, the knee was opened through a medial parapatellar approach, and the medial and lateral sides of the femoral condyle were exposed. A partial capsulotomy and synovectomy were performed. In the animals belonging to group I, MMC (0.2 cc–0.4 mg/mL) was applied in the articular cavity rats before capsulorrhaphy and synovial closures. An identical volume of sterile normal saline was applied in the group II animals, which served as controls. The surgical wound was closed with non-absorbable silk sutures, and external immobilization was not used post-operatively. All rats received weekly (6 weeks) intra-articular both MMC (0.2 cc–0.4 mg/mL) and saline with a 22G needle at the right knee, starting from the day of operation.

Macroscopic evaluation

Animals were euthanized 45 days after surgery by intravenous injection of sodium pentobarbital. The joint was then exposed by skin parapatellar incision. The presence and severity of osteo-capsular adhesion was assessed blinded by the surgeon who performed the surgery (blinded to the treatment group of origin of killed animals) applying the following visual scoring system (Rothkopf DM et al. modified adhesions score) [14]: 0, no adhesions; 1, weak, mild, filmy adhesions that can be eliminated by minimal manual traction; 2, moderate adhesion that can be eliminated by manual traction; and 3, dense fibrous adhesions that must be surgically removed.

Histological evaluation of adhesive tissue

After macroscopic evaluation of adhesions, the knee joints were excised preserving all connective tissues involved in fibrotic adhesive scar formation. The biopsy was fixed in 10% buffered formalin for 1 week and decalcified for 2 weeks. Approximately 7-μm-thick sections were stained with Massons' trichrome stain. The stained tendon sections were evaluated microscopically (Olympus BH-2, Tokyo, Japan) by one independent observer who received the samples without knowing nor treatment group of origin neither which was the treatment on study (double blinded fashion). Presence of fibrous adhesions was accepted as positive if the trichrome positive fibers were seen at the surface of the articular cartilage.

Statistical analysis

The effect of MMC on the reduction in adhesion score in comparison to controls was evaluated by variance analysis (ANOVA). Statistically significant differences were considered when $P < 0.05$. All parameters were also summarized in terms of descriptive statistics (mean and standard error).

Results

The surgery was well tolerated by all 20 animals. The MMC was easily applied, covered uniformly and adhered well to all injured surfaces. At 14 days post-surgery, one animal in the control group and one animal in the treated group showed mild infection with edema and treated well with intravenous antibiotic therapy without any problem.

After the follow-up period, a significant reduction in the adhesion score was observed in MMC-treated group in comparison to untreated controls (Table 1). Macroscopically thicker and more fibrous adhesions, often associated with swelling and macroscopic inflammation, developed between the surface of the femoral condyle and the adjacent parapatellar area in the control group. The only three slight adhesions observed in the MMC-treated group were filmy, transparent and easily dissected.

Histological evaluation showed no foreign body reaction in the MMC-treated group, and only a slight macrophage population was evident. Articular cartilage demonstrated a regular morphology with its surface and cartilage cells. The cartilage cells had regular isogenous alignment and morphology (Fig. 1). Substantial fibrosis was seen in the control group, and adhesions appeared as thick and dense fibers with a predominant population of mature fibroblasts. Articular cartilage had shown degenerative local sites ranging from minimal to moderate. Although the surface of the cartilage was smooth in most areas, some irregular surfaces were present. In those sites, the hypocellularity and hypertrophy of the cartilage was prominent (Fig. 2).

Discussion

The most important finding of the present study was that arthrofibrosis after knee surgery can be reduced by applying biochemical agent. This agent diminished abnormal proliferation of a fibro-adhesive scar around the connective tissues of a joint that can lead to loss of motion, stiffness and pain. It also suspended the need for a second surgery and hard physical therapy sessions for treatment.

Moreover, in the case of joint adhesions, along with articular pain, one of the main problems that can severely affect daily activities is the loss of joint mobility [1, 2]. The presence of scar tissue near the cruciate ligaments and tibial–femoral joint is thought to be primarily responsible for loss of extension, with flexion loss resulting from fibrous tissue formation around the patellar–femoral articulation and subsequent adherence of the patella to the trochlear groove [6].

Table 1 Osteo-capsular adhesion in rabbit knee after capsulotomy and synovectomy

Animals	Macroscopic visual scoring system	
	Group 1	Group 2
1	0	3
2	1	3
3	0	2
4	0	2
5	0	3
6	0	3
7	1	2
8	0	3
9	0	2
10	1	3
Average	0.3; SD: 0.5	2.6; SD: 0.5
P value	0.0124	

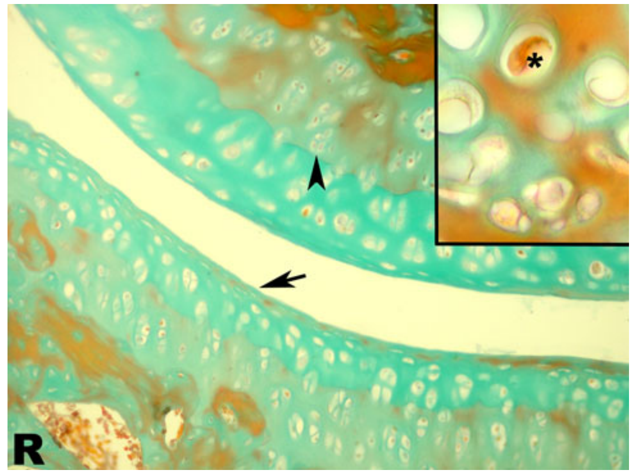

Fig. 1 The cartilage cells had regular isogenous alignment and morphology. The articular cartilage surface with regular contour (*arrow*) and isogenous cells (*arrowhead*), the hyaline cartilage cells with their rounded shapes (*inset asterisks*) with Masson's Trichrome stain (×200, insets ×1,000)

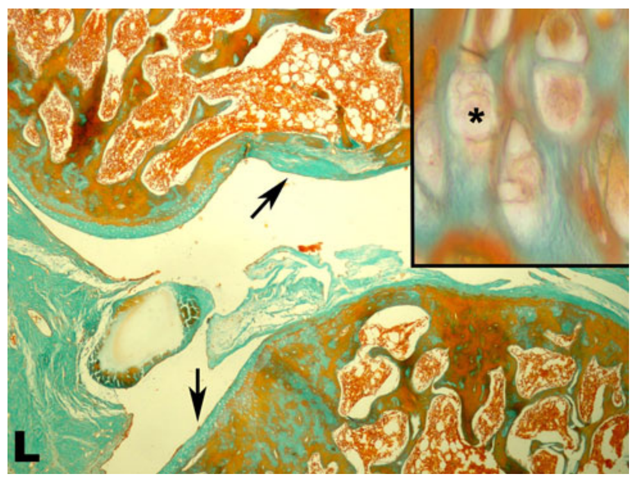

Fig. 2 The articular cartilage surface with irregular contour in some regions (*arrows*), note the hypocellularity and hypertrophy of cartilage cells (*inset asterisks*) with Masson's Trichrome stain (×100, insets ×1,000)

In recent past, several approaches have been suggested to minimize the knee fibro-adhesive scar formation, including early mobilization and pharmacological therapies such as administration of anti-inflammatory corticosteroid and non-corticosteroid agents. Anti-inflammatory therapies have a role in minimizing the formation of adhesions following injury or surgery to the knee. Some authors have used intravenous corticosteroids during the immediate post-operative period [1, 2].

Recently, the use of anti-adhesion barriers such as oxidized regenerated cellulose and chemically modified hyaluronan and carboxymethylcellulose all based on biocompatible and absorbable polymers has been proposed to reduce adhesive scar in a variety of surgical procedures [2, 9, 11, 16]. These biomaterials have been applied in order to keep connective adjacent healing surfaces separated. However, poor outcomes are often obtained [2, 9, 11, 16]. The use of polymeric solutions such as gelatincarbohydrate has shown a reduction in post-operative adhesions in experimental tendon and peripheral nerve surgery [2, 12]. However, the results from human clinical trials have been conflicting or involved adverse events [10]. In this study, we investigated the use of Mitomycin C (MMC), as a possible biochemical agent reducing fibrosis formation administered as injection during and after knee surgery.

MMC is produced by Streptomyces cuspidatus and initially used as an aminoglycoside antibiotic and anti-neoplastic agent [3, 8, 16]. Its mechanism of action is through cross-linking of DNA. MMC has been investigated for the last 20 years for its ability to reduce scar formation. Its proposed mechanism of action is through prevention of fibroblast proliferation, a major step in scar formation, by blocking DNA synthesis by cross-linking DNA [3, 8]. The anti-proliferative effect of MMC on cultured human fibroblasts is observed at lower concentrations, whereas cytocidal activity against these cells is seen at higher concentrations, at which the drug starts to inhibit ribonucleic acid and protein synthesis [3, 8].

With regard to the animal model selected, the surgery was well tolerated by all animals. The MMC was easily applied, covered uniformly and adhered well to all injured surfaces. We were also able to make intra-articular injection weekly. At the end of the follow-up time, the high incidence (100%) of adhesions found in the control group indicates that experimental surgical procedure used was adequate for this type of study.

In the presented study, a significantly lower incidence of animals showing adhesions was seen in MMC-treated group with respect to the control group. Macroscopically thicker and more fibrous adhesions developed between the surface of the femoral condyle and the adjacent parapatellar area in the control group, which was a known place for adhesion formation [2, 6]. The only three slight adhesions observed in the MMC-treated group at the same area were filmy, transparent and easily dissected.

Histological evaluation showed substantial fibrosis in the control group, and adhesions appeared as thick and dense fibers with a predominant population of mature fibroblasts. Conversely, no foreign body reaction was seen in the MMC-treated group, and only a slight macrophage population was evident, and cartilage demonstrated a regular morphology with its surface and cartilage cells. This proved that MMC is not toxic for the cell lines at the given concentration. The reason for this is we used low doses of MMC.

Our study had some limitations. First, the role of concentration and molecular weight of MMC injected in knee joint was less clear, and further elucidation using a dose titration evaluation scheme would need to be completed. Second, we did not use gross range of motion for the evaluation of knee adhesion. Gross range of motion was used as an indirect measure of the level of arthrofibrosis [2]. For that reason, we decided that range of motion was not an adequate indicator to assess the level of tendon adhesion in this model.

Conclusions

In conclusion, MMC demonstrated efficacy in preventing adhesions after knee surgery, and all the parameters monitored showed consistent statistically significant improvement. In this in vivo study, intra-articular administration showed no unsafe action, confirming the elevated safety profile and biocompatibility of the device observed in previous clinical trial in different surgical procedures. Therefore, MMC may be considered a promising biochemical agent to control scar tissue formation after open and arthroscopic knee surgery.

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