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TGF-β1 as a prognostic factor in the process of early osteoarthrosis in the rabbit knee

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Summary

Objective: To assess changes in knee joint fluid concentrations of transforming growth factor-β1 (TGF-β1) and proteoglycan (PG) fragments during the early course of post-traumatic osteoarthrosis (OA) after meniscectomy in the rabbit knee, and to ascertain whether the concentrations of these substances shortly after operation could be used as prognostic markers for the OA process.

Design: In 15 rabbits with medial meniscectomy in one knee and a sham operation in the other knee, synovial lavage fluid samples were taken repeatedly, before operation, every third week post-operatively until 12 weeks, thereafter every sixth week, and at death. Five rabbits each were killed at 13, 25 and 40 weeks. Synovial lavage fluid samples from five non-operated rabbits served as controls. At death, two histological scores were formed that characterized the highest (MAX) and the overall (ALL) degree of OA changes in each joint.

Results: TGF-β1 and PG fragment concentrations in synovial lavage fluid correlated highly (R=0.81, P<0.001). Both OA scores were higher in meniscectomized than controls (P<0.05). The synovial lavage fluid concentration of TGF-β1 at 3 weeks, but no other time point, correlated to the histological scores (ALL, R=0.58; MAX, R=0.52; P<0.001).

Conclusion: Higher concentrations of TGF-β1 in synovial lavage fluid early after surgery seemed indicative for the later development of more severe OA changes in contrast to lower concentrations. The association between TGF-β1 and the changes found later in the cartilage was underlined by the high correlations between this substance and PG fragment concentrations in synovial lavage fluid at all time points.
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Key words: Meniscectomy, Transforming growth factor-β1, Proteoglycans, Articular cartilage.

Introduction

The initiation of osteoarthrosis (OA) involves a complex interplay between cytokines, growth factors, matrix proteins and tissue metalloproteinases in articular cartilage and the underlying bone[1,2]. In osteoarthrotic cartilage, the collagen–proteoglycan network in the extracellular matrix is disturbed, articular chondrocytes show an increased proteoglycan (PG) and collagen synthesis, newly synthesized PGs are degraded at higher rates than normal[3,4], and increased amounts of cartilage molecule fragments are released into joint fluid[5]. A weak positive correlation was found between osteoarthrotic cartilage changes and concentrations of PG fragments in knee joint fluid of rabbits after various procedures to menisci and ligaments[6]. The PG synthesis in articular chondrocytes is influenced by transforming growth factor-beta 1 (TGF-β1)[7]. TGF-β is also involved in osteoblast formation, bone remodeling[8,9] and in osteophyte induction. Repeated TGF-β1 injection in the mouse knee joint induced an inflammatory reaction, and finally formation of osteophytes, the size and location of which appeared to correlate with the spatial distribution and degree of cartilage damage[10].

Animal models of experimentally induced joint cartilage disease have been frequently employed, using a variety of techniques[11,12]. They allow a detailed investigation of the early mechanisms of pathogenesis. Meniscectomy has been commonly used as an OA model in rabbit[13,14], dog[15] and sheep[16]. Similar cartilage changes, formation of osteophytes and subchondral bone remodeling as in human OA have already been found some weeks after meniscectomy[13].

At early stages of OA, before radiographic changes confirmed the joint disease, increased joint fluid concentrations of cartilage and bone matrix molecules were noted in joint fluid, blood and urine[17,18]. Increased joint fluid concentrations of these substances were also found in knees with established radiographic changes[5,17], but it has not been demonstrated that the concentrations of these markers at an early disease stage were prognostic for the severity of the disease at later stages.

We recently demonstrated in rabbits a physiologic decrease of the knee joint fluid concentrations of TGF-β1 and PG fragments during maturation. The concentrations of these substances in joint fluid were also found to have increased shortly after creation of an osteochondral defect. The higher TGF-β1 concentrations in young and adolescent rabbits compared with the adult ones, both before and shortly after the operation, coincided with a

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better healing capacity of the defect, but at the same time a higher susceptibility of the cartilage adjacent to the defect to osteoarthrotic change[19]. We now hypothesize that joint fluid concentrations of TGF-β1 and PG fragments at an early stage after meniscectomy will be indicative for the cartilage and bone changes that will eventually develop after this procedure.

Thus, the purpose of the present study was to continuously assess changes in TGF-β1 and PG concentrations in knee joint fluid during the early course of post-traumatic OA after meniscectomy in the rabbit knee, and to ascertain whether the concentration of TGF-β1 and/or PG fragments, at an early phase after operation, are prognostic for the knee joint changes which develop later.

Materials and methods

Fifteen over 4 months old (range 19–62 weeks) female New Zealand white rabbits weighing between 3.3 and 5.1 kg, with medial meniscectomy in one knee and a sham operation in the other knee, underwent operation. Immediately before operation, every third week after operation until 12 weeks, thereafter every sixth week, and at death, synovial lavage fluid was sampled from both knees. When the time of death coincided ±1 week with the planned joint fluid sampling, we took merely joint fluid at death. Five rabbits each were killed after 13, 25 and 40 weeks. Additionally, five non-operated rabbits served as controls, from which synovial lavage fluid samples were aspirated on a single occasion. The experiment was approved by the local Ethics Committee and the guidelines for animal use and care were followed.

SYNOVIAL LAVAGE FLUID SAMPLING AND SURGICAL PROCEDURE

The synovial lavage fluid sampling and the operation were performed under sterile conditions using intravenous anesthesia with ketamine (Parke-Davis, S.A., Barcelona, Spain) and Xylazinchloride (Bayer, Mannheim, Germany) (15 mg/kg and 1.5 mg/kg, respectively). After shaving and disinfection of the area around the knee joint, a 21G2 needle (Microlance, Becton Dickinson, Dublin, Ireland) was inserted from the medial side of the patellar tendon into the anterior compartment of the knee at a slightly flexed knee position. The knee was extended, and the tip of the needle was placed into the suprapatellar bursa. One milliliter of sterile 0.9% NaCl solution was injected. The knee was moved 20 times through a full range of motion, and the joint fluid aspirated. Keeping the needle in place, the above procedure was repeated. The two samples from each knee were combined, the volume measured, and stored at −20°C until analysed. After the joint fluid sampling the medial meniscus was removed in all right knees through a medial skin and two capsular incisions, anterior and posterior to the medial collateral ligament. In the left knees, similar skin and capsular incisions were made but the meniscus left intact (sham operation). At the end of each procedure the joint was irrigated with physiological saline and some drops of antibiotics (Oxytetracyclinehydrochloride, Pfizer, Amboise, France), and the wound closed in layers after surgery. The same antibiotics as used locally were given intravenously (10 mg/kg) at the end of operation. Post-operatively, free movement was allowed within the separate cages (area 0.5 m^2). The wound healing was observed daily until healing and full functional recovery. The repeated synovial lavage fluid sampling was done under general anesthesia and sterile conditions as described above. At the indicated time the animals were killed with an overdose of Pentabarbituralnatrium (Apoteksbolaget, Umeå, Sweden). Immediately after death both hind limbs were disarticulated at the hip joint. The soft tissues were removed but the knee joint capsule and ligaments kept intact. The femur was cut at the level of the trochanter major and the tibia above the ankle joint. The lower part of the fibula was removed from its synostosis to the tibia. The specimens were wrapped in saline soaked gauze and kept refrigerated. Before the final histological preparation, which was done within 36 h of death, the knees were examined radiographically, and mineral density of the subchondral bone was assessed. The results of the last two examinations are described in detail elsewhere[14].

HISTOLOGICAL EVALUATION AND ANALYSIS

Both knees of operated and control animals were opened and dissected. The gross appearance of articular cartilage, any formation of osteophytes, and the extent of resection of the meniscus were recorded on a protocol. The femur was cut above the trochlear groove and condyles, the tibial shaft below the tibial insertion of the medial collateral ligament. The femoral condyles and tibial plateau were fixed separately in 4% paraformaldehyde solution for 1 week, and then decalcified in 22% formic acid for 5–16 weeks. After decalcification sagittal sections of 1–2 mm thickness were cut from the middle of the medial and lateral femoral condyles (Fig. 1). The tibial plateau was divided sagittally along its midline and frontal sections of 1–2 mm thickness each were cut from the medial tibial plateau at anterior, central and posterior regions (Fig. 1). From the lateral tibial plateau a frontal section was taken from the central part only (Fig. 1). Each of the above-described six different specimens of one knee was embedded separately in paraffin, sections of 5 μm thickness were cut and stained with alcian-blue/periodic acid Schiff and safranin-O. Cartilage and bone changes were evaluated by the modified Mankin score according to van der Sluijs[20]. The score describes changes in cartilage structure and matrix staining, and cellular abnormalities, and it ranges from 0 (normal cartilage) to 13 (worst case of OA). A minimum of five sections from different levels was used for evaluation of each tissue block. According to the original publication by Van der Sluijs[20], the score has a mean intraexaminer variation of 0.4±1.5 points and an interexaminer variation of 1.3±1.7 points (mean diff.±S.D.)[20]. In the present investigation it had a mean interexaminer variation of 0.4±1.8 points (mean diff.±S.D.)[14], when one experienced examiner (KM) and two less experienced examiners (BA, AF) evaluated all samples independently. For the comparison with the synovial lavage fluid concentrations, the values from the experienced examiner (KM) were used.

For comparisons with the concentrations of TGF-β1 and PG fragments in synovial lavage fluid two histological scores were formed, one that characterized the overall degree of osteoarthrosis (ALL) and another that represented the highest degree of cartilage disease (MAX) in each joint. For the overall score, each of the six individual sections of the joint (Fig. 1) was scored at two locations, on the femur at anterior and posterior locations (Fig. 2), and on the tibia at central and peripheral locations (Fig. 2). The

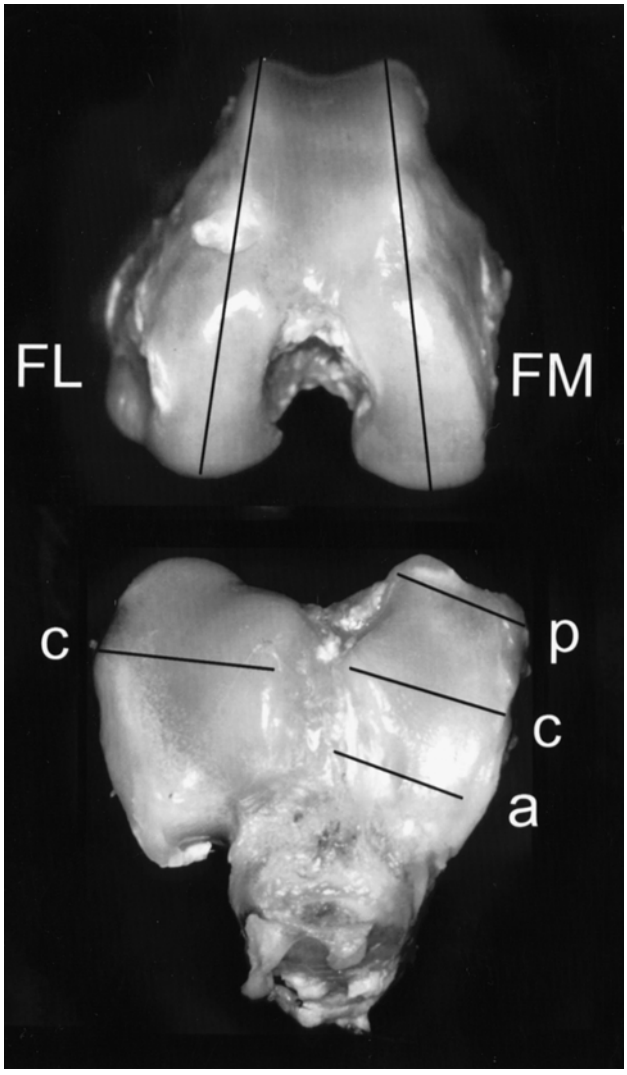

Fig. 1. Six different sites of the rabbit knee joint were evaluated histologically and are represented by straight lines. On the femur the medial and lateral condyles were evaluated at one location each, on the tibia the medial and lateral plateaux were evaluated at a central location. In addition, anterior and posterior locations were evaluated on the medial tibial plateau. FM: femur medial; FL: femur lateral; a: anterior; c: central; p: posterior.

anterior location on the femur represents the region that mainly is in contact with the patella during knee flexion and non-weight-bearing, the posterior one is loaded during gait. On the tibia, the central location represents the area of direct contact between the femoral condyle and tibial plateau, at the peripheral one the meniscus is interposed. For the medial and lateral femoral condyles and the lateral tibial plateau, respectively, a combined score was formed by adding the values of the two locations and dividing them by 2. For the medial tibial plateau, which consisted of three sections with two locations each, all six values were added to a combined score and divided by 6. Finally the mean values of each femoral condyle and tibial plateau (N=4) were added and divided by 4, forming the overall score. The maximum score was formed by taking the highest score value for each joint representing the highest degree of the disease.

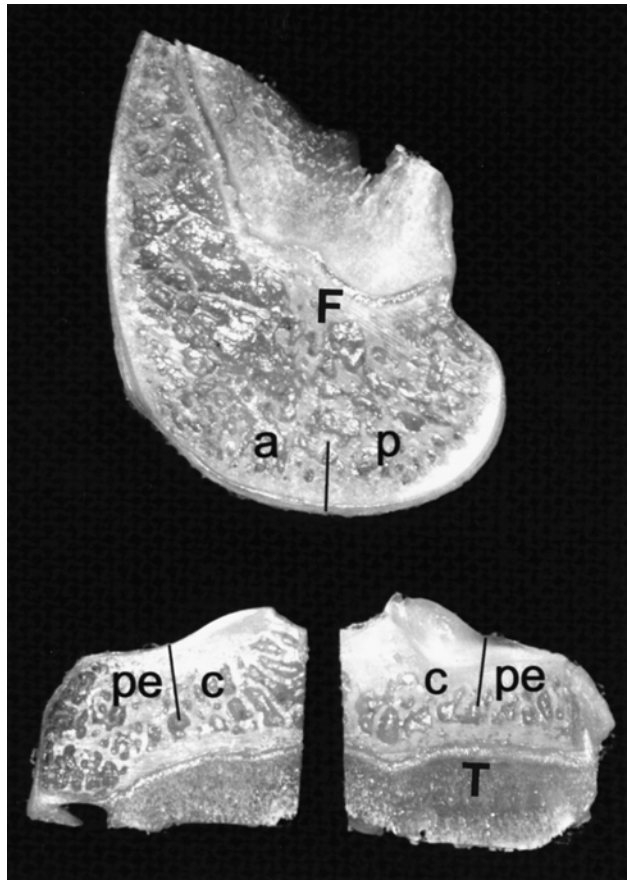

Fig. 2. The histological sections were scored separately at anterior and posterior locations on the femoral samples, and on central and peripheral locations on the tibial samples. F: femur; T: tibia; a: anterior; p: posterior; c: central; pe: peripheral.

MEASUREMENTS OF TGF-β1 AND PG FRAGMENT CONCENTRATIONS IN SYNOVIAL LAVAGE FLUID

The concentration of TGF-β1 in synovial lavage fluid samples was determined by using the Quantikine® TGF-β1 immunoassay according to the protocol included in the kit (Quantikine® R&D Systems, Inc., U.S.A.). The optical density of each well was determined using a spectrophotometer (Anthos HT3, Anthos Labtec Instruments, Austria) set to 450 nm with correction of 550 nm. Recombinant human TGF-β1 was used as standard, with serial dilutions of 1.95 pg/ml and 1000, 500, 250, 125, 62.5, 31.2 and 0 pg/ml respectively.

The PG concentrations in synovial lavage fluid were analysed by precipitation of sulfated glycosaminoglycans containing proteoglycan fragments with Alcian blue[21]. Chondroitin sulfate (Sigma C4384) was used as standard.

STATISTICS

We used the Student's paired *t*-test to compare the amount of aspirated synovial lavage fluid and concentrations of TGF-β1 and PG fragments between left (sham-operated) and right knees (meniscectomized) of the same animal, and for comparisons of synovial lavage fluid volume before operation and at time of death. The Student's unpaired *t*-test was used to compare the synovial lavage

Table I
Synovial lavage fluid volume of meniscectomized and sham-operated knees, before operation and at death (mean±1 S.D.)

Group	*N*	Pre-op (ml) (Me)	At death (ml) (Me)	Pre-op (ml) (Sh)	At death (ml) (Sh)
13 week	5	1.4±0.3	1.5±0.1	1.1±0.2	1.3±0.3
25 week	5	1.4±0.3	1.5±0.1	1.4±0.1	1.4±0.1
40 week	5	1.4±0.2	1.5±0.1	1.3±0.2	1.5±0.2
Controls	5		1.5±0.1		1.2±0.3

Me: Meniscectomized knees; Sh:sham-operated knees. Groups with 13, 25 and 40 weeks follow-up. There were no significant differences between the groups.

Table II
Combined histological scores at the different follow-up times (median, range)

Group	*N*	ALL (Me)	MAX (Me)	ALL (Sh)	MAX (Sh)
13 weeks	5	2 (1.5–3.5)*†	8 (6–12)*	0.5 (0–1.5)	4 (0–8)
25 weeks	5	2.5 (1.5–3.5)*	10 (7–13)*	1.5 (0.5–2)*	5 (2–9)*
40 weeks	5	2.5 (1.5–3)*†	8 (7–13)*	0.5 (0–2.5)*	4 (3–12)*
Control	5	0 (0–0.5)	1 (1–2)	0 (0–0.5)	1 (0–5)

Me: Meniscectomized knees; Sh: Sham-operated knees; ALL: Overall score; MAX: Maximum score; * higher scores than controls at specific time point ($P<0.01$); † higher scores than paired sham-operated knees at specific time point ($P<0.04$).

fluid concentrations (TGF-β1 and PG fragments) between either the right or left knee of the operated animals and the controls, and the synovial lavage fluid volume between operated and control knees. To compensate for the increasing probability of type I error during multiple comparisons using *t*-tests, the *P*-level was adjusted by dividing the defined *P*-value ($P<0.05$) for the study by the number of comparisons (up to eight comparisons) (Bonferoni correction). A significance level of lowest $P<0.006$ was required, depending on type of comparison. For correlations between synovial lavage fluid concentrations (TGF-β1 and PG fragments) and the histological scores (ALL and MAX) the Spearman rank correlation for non-parametric values was chosen. The Pearson's test for parametric data was used for correlations between TGF-β1 and PG fragment concentrations. Multivariate analysis of variance (MANOVA) was used to determine the overall effect of time point after operation and surgical procedure. Statistical significance was defined as $P<0.05$. All calculations were performed using commercially available software (STATISTICA; Statsoft Inc®, Tulsa, OK).

Results

Two rabbits were replaced because of accidental death during anesthesia. In total seven samples of TGF-β1 concentrations in synovial lavage fluid could not be determined because of gross contamination with fresh blood, which makes an accurate analysis of this substance impossible. However, we did not specifically measure the amount of hemoglobin in the samples. The amount of aspirated synovial lavage fluid from right and left knees was similar in all groups before and after operation (Table I). Furthermore, there was no difference in amount of synovial lavage fluid between the operated and control knees.

GROSS APPEARANCE

The medial meniscus had been excised completely except for an occasional remnant of its posterior horn and attachment ligament. Meniscal regrowth was not noted. The tibial cartilage showed fibrillation in all meniscectomized knees, and in all except one osteophytes had formed at the periphery of the tibial plateau. Likewise, at the medial femoral condyle nine of the meniscectomized knees showed fibrillation, and five of them osteophytes. The lateral femoral condyle did not have any macroscopic changes. Four sham-operated knees had cartilage fibrillation on the medial tibial plateau, and three of them a tibial osteophyte but no change on the femur.

HISTOLOGY

Meniscectomy induced cartilage changes typical for osteoarthrosis, which progressed over time on the posterior aspect of the medial tibial plateau ($P<0.009$). The results of the specific locations are presented in detail elsewhere[14]. The overall histological score (ALL) and the maximum score (MAX) were significantly higher in meniscectomized knees than in controls at all follow-up times ($P<0.01$). There were no significant differences between the different meniscectomized groups. Only at 25 and 40 weeks did the sham-operated knees have higher scores (ALL, MAX) than the controls ($P<0.01$). In the operated animals, the meniscectomized knees had a higher overall score than the sham-operated at 13 and 40 weeks after operation ($P<0.04$) (Table II). There was no difference between right and left control knees in histological score.

CONCENTRATIONS OF TGF-β1 AND PG FRAGMENTS IN SYNOVIAL LAVAGE FLUID

In the whole operated material ($N=15$ animals, 30 knees) there was a significant effect of time interval of

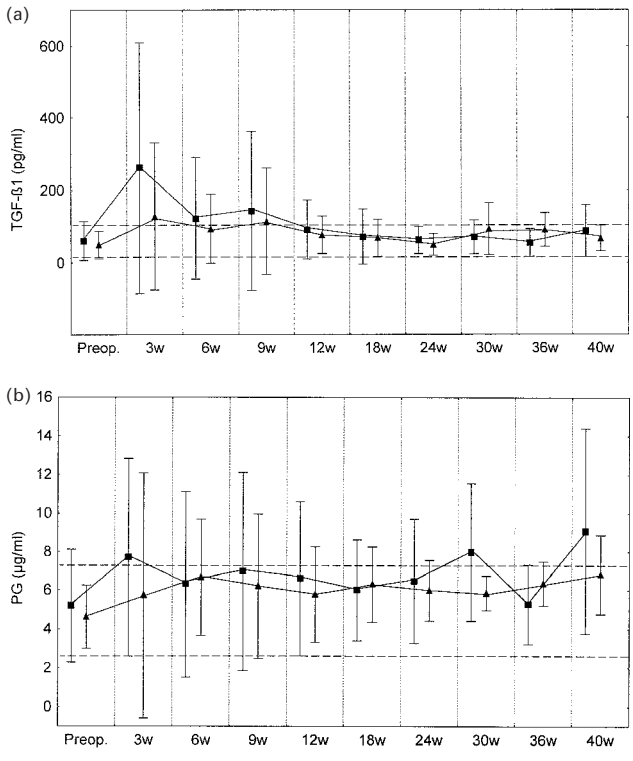

Fig. 3. The changes over time concerning TGF-β1 (a) and PG fragment (b) synovial lavage fluid concentrations from meniscectomized and sham-operated knees. Data represent mean ±1 S.D. of the meniscectomized knees (■) and sham-operated knees (▲). The area between the two dashed lines denotes the mean±1 S.D.-value for the pre-operative samples.

synovial lavage fluid sampling on PG fragment and TGF-β1 concentrations during the first 6 and 12 weeks after surgery, respectively (MANOVA, $P<0.05$). However, the choice of surgical method had no effect. In contrast, at separate analysis of the animals with a 40 week follow-up ($N=5$ animals, 10 knees), both time after surgery and the choice of surgical method had a significant influence on the synovial lavage fluid concentrations of both substances (TGF-β1 and PG) during the whole observation period (MANOVA, $P<0.05$).

At specific subgroup analysis using paired *t*-tests, there was no significant difference in synovial lavage fluid concentrations of TGF-β1 or PG fragments between specific time points in either meniscectomized or sham-operated knees. Further, there was no difference between left and right knees at any specific time point (paired *t*-test), nor was there any difference between controls and operated knees (unpaired *t*-test) [Fig. 3(a),(b)].

CORRELATIONS BETWEEN THE TGF-β1 AND PG FRAGMENT CONCENTRATIONS IN SYNOVIAL LAVAGE FLUID AND THE HISTOLOGICAL SCORES

At all time points TGF-β1 and PG fragment concentrations in the synovial lavage fluid were highly correlated ($R=0.81$–0.91, $P<0.001$) [Fig. 4(a)–(d)].

Only the concentrations of TGF-β1 fragments at 3 weeks were correlated to the overall (ALL, $R<0.58$, $P<0.001$) and maximum histological scores (MAX, $R=0.52$, $P<0.001$) as evaluated at time of death [Fig. 5(a),(b)]. No such correlation existed between the final histological scores

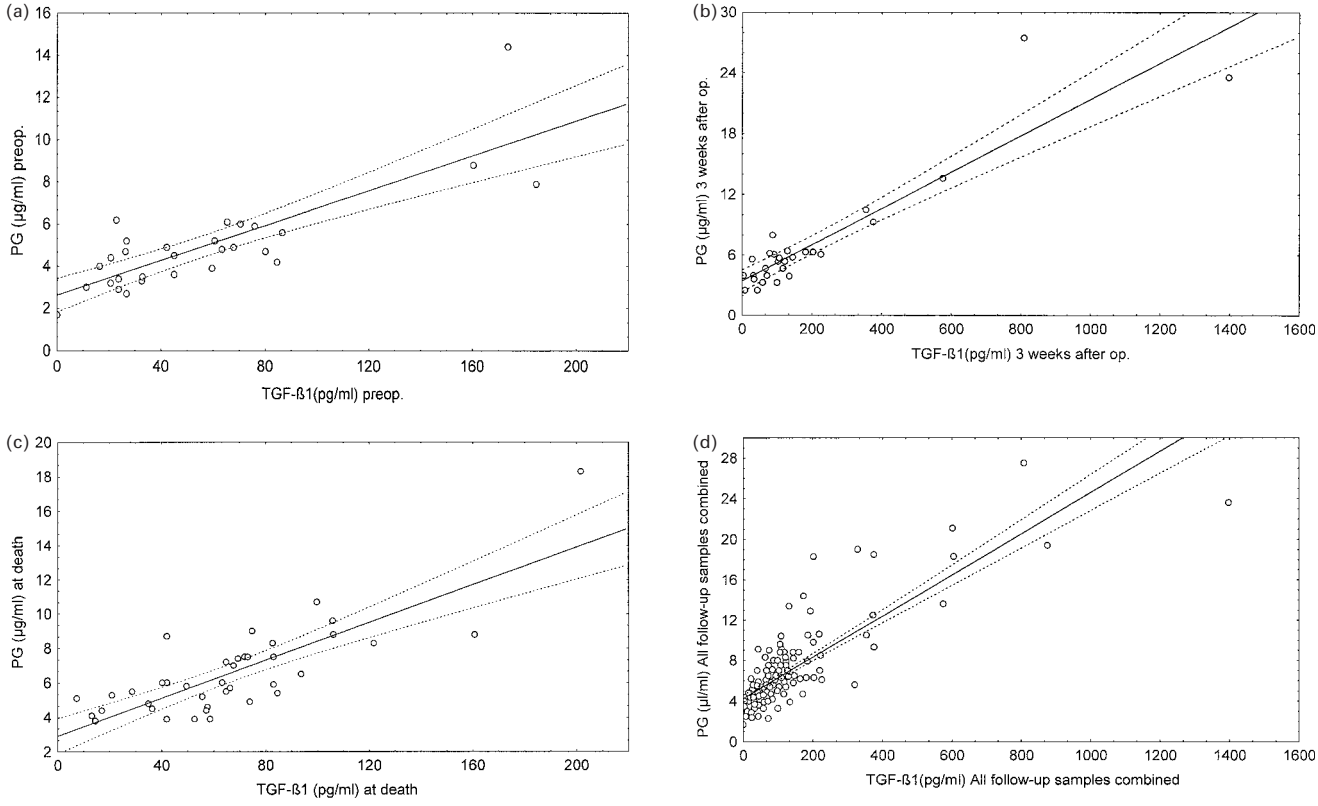

Fig. 4. Correlations between the TGF-β1 and PG fragment concentrations in synovial lavage fluid of operated animals (meniscectomized and sham-operated), (a) before operation ($R=0.82$, $P<0.001$), (b) at 3 weeks ($R=0.91$, $P<0.001$), (c) at the time of death ($R=0.81$, $P<0.001$), and (d) all follow-up values combined ($R=0.81$, $P<0.001$).

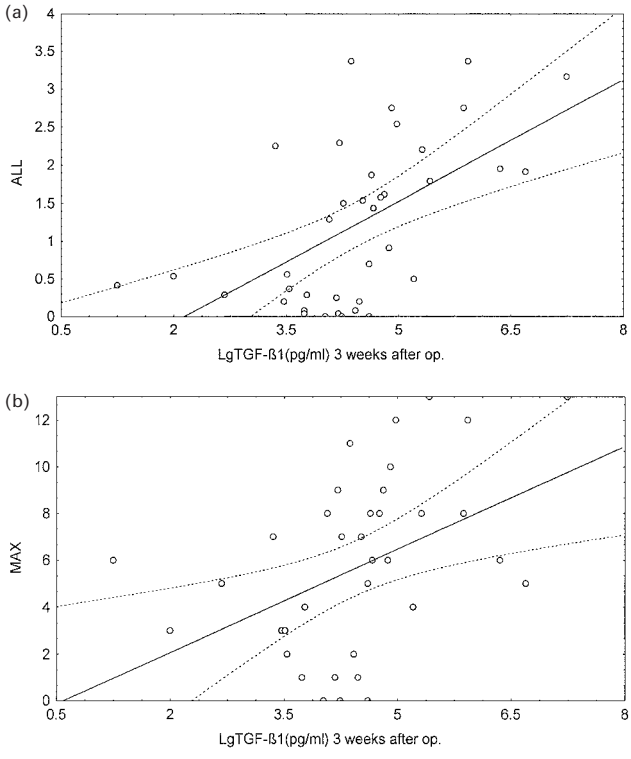

Fig. 5. Correlations between the synovial lavage fluid concentrations of TGF-β1 (logarithmic) at 3 weeks after surgery and (a) the overall (ALL) histological score (R=0.58, P<0.001), and (b) the maximum (MAX) histological score (R=0.52, P<0.001).

and PG fragment concentrations in synovial lavage fluid at any time point.

Discussion

High concentrations of TGF-β1 in synovial lavage fluid at an early stage after surgery (3 weeks) seemed indicative for the later development of more severe OA changes in contrast to lower concentrations. Furthermore, the association between TGF-β1 and the changes found later in articular cartilage was underlined by the high correlations between this substance and PG fragment concentrations in synovial lavage fluid at all time points.

Hemarthrosis after both surgical procedures was probably an important source for the increase in TGF-β1 concentration in synovial lavage fluid at 3 weeks, since TGF-β1 is found in great amounts in platelets[8]. Hemarthrosis alone has been shown to induce changes in the articular cartilage, typically for mild OA[22]. The injuries to cartilage by the surgical procedure and joint fluid sampling as shown in detail elsewhere[14], may be another source, as articular cartilage beside bone has been shown to contain TGF-β1[23]. The post-operative inflammatory and repair process and the initial resorption in subchondral bone that occurs in the early OA process[14] may lead to a further TGF-β1 generation, activation and release[24].

In vitro studies indicate that TGF-β1 affects skeletal tissues by inducing proliferation and differentiation of osteoblasts, and synthesis of type II collagen[25] and cartilage proteoglycans[26,1]. The signal a chondrocyte receives from a soluble regulator such as TGF-β1 depends, of course, on several factors, such as the presence of regulators, the level of cell differentiation[27], mechanical stimuli[28] and the presence of specific receptors[29]. It is suggested that TGF-β's complex role is related to a shift in receptor expression that occurs in the differentiation process in OA chondrocytes[30]. Injections of TGF-β1 in murine knee joint have shown to increase the synthesis of proteoglycans, and at the same time to induce osteophytes[31]. TGF-β1 has indeed been shown to be a dominant factor in osteophyte formation[10], and in the bone tissue of OA patients an increase in TGF-β concentration was also noted[32]. These processes may have initiated the conspicuous osteophyte formation which we noted already at 13 weeks after meniscectomy. TGF-β acts also as a regulator of the balance between cartilage matrix metalloproteinases and their inhibitors and, therefore, may have the potential to contribute to the pathological process in the cartilage[33]. Degradation of proteoglycans has been found to correlate with increased expression of various metalloproteinases at the articular surface in OA[4].

In patients with various injuries to their knee ligaments and menisci, increased concentrations of PG fragments and other markers of cartilage metabolism have been noted in the knee joint fluid soon after trauma and persisted for years[18]. A weak positive correlation between increased concentration of PG fragments in joint fluid and the degree of cartilage destruction was shown in a rabbit experiment with various injuries to ligaments and menisci[6]. In this study we demonstrated some increase in PG fragment synovial lavage fluid concentrations after isolated meniscectomy or a sham operation. Likewise, when an osteochondral defect was created in a similar rabbit model, increased levels of proteoglycan fragments and TGF-β1 were found in joint fluid 3 weeks after surgery[19].

However, although the surgical trauma and following hemarthrosis may have been more pronounced after meniscectomy than after a sham operation, there was no significant effect of choice of surgical method on TGF-β1 or PG fragment concentrations during the first 12 weeks after trauma. Probably the differences were not large enough to be picked up, especially since the concentrations of both substances varied largely and the observations were rather few. Though samples with gross blood contamination were excluded from analysis, some of the variability of TGF-β1 concentrations may be attributed to an uncontrolled contamination by smaller amounts of fresh blood thanks to the procedure of synovial lavage fluid sampling.

Also, the representativeness of synovial lavage fluid samples compared to undiluted samples may be discussed. With the lavage technique, when properly performed, joint fluid is evenly mixed with a rather large amount of saline. It should thus give the average concentration of a marker in joint fluid, albeit highly diluted. However, the dilution process may add to errors in measurement. Undiluted samples undoubtedly contain higher marker concentrations,[34] but at the same time may not represent their average concentrations in the whole joint fluid, since there may be local differences. Nevertheless, in a long-term follow-up study of patients with previous meniscectomy, undiluted knee joint fluid samples showed similar patterns for PG fragment concentrations as synovial lavage fluid samples.[35] Another reason for the lack of difference between differently treated knees within an individual animal may be that joint fluid concentrations of various markers from left and right knees may influence each other and therefore diminish the differences which otherwise would occur due to two different types of operations. Dahlberg *et al.*[36] showed that the uninjured knee in patients with unilateral knee injury showed abnormally high

values of PG fragment concentrations and other markers; therefore values from non-operated individuals are necessary. Nevertheless, higher TGF-β1 concentrations at 3 weeks were to a certain degree indicative of higher OA changes at death, and meniscectomy resulted in more pronounced cartilage changes than a sham operation.

Meniscectomy has shown *in vitro* to increase the stress on the tibial plateau by a marked decrease in contact area between the opposing joint surfaces[37,38]. In addition to the initial trauma, this stress may be harmful to the articular cartilage and bone in the long term. However, the synovial lavage fluid concentrations of TGF-β1 and PG fragments, respectively, were similar after meniscectomy or a sham operation during the whole observation time of 40 weeks. The correlation between initial TGF-β1 values and the cartilage status at time of death (but at no other time point), although low, may suggest that the initial trauma, rather than the alteration in knee joint mechanics, was decisive for the development of early changes in post-meniscectomy osteoarthrosis. A similar conclusion was drawn when bone density of the proximal tibia was measured in the same animals[14]. Here, an increase in bone and cartilage changes was noted on the medial tibial plateau, despite a corresponding subchondral bone mineral density which was below normal during the whole observation time. Thus, an increase in bone mineral density owing to stress increase was not observed during the first 40 weeks after meniscectomy, and subsequently increased bone stiffness because of mechanical joint alterations therefore can not be blamed primarily for the development of cartilage damage during the early course of OA. However, it cannot be excluded that part of the TGF-β1 release as measured after 3 weeks and later was due to the mechanical joint load alterations revised by the lack of a meniscus. Mechanical stimuli via stimulation of TGF-β1 may play a role in the initiation of OA after meniscectomy[28].

According to our results, post-operative TGF-β1 concentrations in synovial lavage fluid seem indicative for the process of articular cartilage degeneration in post-traumatic OA. It may be suggested that management of the early inflammatory process after trauma may be beneficial for the protection of joint cartilage after intraarticular injuries.

Acknowledgments

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