Effect of Radiosynovectomy with Holmium-166 Ferric Hydroxide Macroaggregate on Adult Equine Cartilage

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ABSTRACT. ***Objective.*** To analyze the effect of radiosynovectomy with holmium-166 ferric hydroxide macroaggregate (^{166}Ho-FHMA) on articular cartilage in 6 adult horses.

Methods. Arthritic changes and mechanical properties of articular cartilage were evaluated with arthroscopy and postmortem microscopic analyses. Glycosaminoglycan content was measured by safranin-O staining combined with digital densitometry, uronic acid analyses, and dimethylene blue binding assay. ^{35}S-sulfate labeling and autoradiography were used to localize proteoglycan synthesis and to characterize proteoglycan structures using SDS-agarose gel electrophoresis. Northern hybridizations were performed to measure the mRNA levels for aggrecan and pro-α_1(II) collagen in cartilage samples.

Results. Histological signs of degeneration were present in the articular cartilage of both control and radiosynovectomized equine joints. Radiosynovectomy did not aggravate degenerative changes or significantly alter the matrix glycosaminoglycan content. A slightly decreased size of proteoglycan monomers was observed 2 months after ^{166}Ho-FHMA radiosynovectomy. Tissue analysis of extracted proteoglycans revealed lower ^{35}S incorporation after radiosynovectomy, but corresponding changes could not be observed in aggrecan mRNA levels. Transient downregulation of pro-α_1(II) collagen mRNA transcription was observed 5 days after ^{166}Ho-FHMA radiosynovectomy.

Conclusion. ^{166}Ho-FHMA treatment did not markedly affect the composition or morphology of adult articular cartilage showing mild degeneration. However, minor degradation of proteoglycan monomers and transient downregulation of pro-α_1(II) collagen mRNA were observed. (J Rheumatol 2004;31:321–8)

Key Indexing Terms:

RADIOSYNOVECTOMY OSTEOARTHRITIS EQUINE
ARTHROSCOPY COLLAGEN TYPE II PROTEOGLYCAN

An inflamed synovium is an important source of proinflammatory mediators such as interleukin 1 and tumor necrosis factor-α that have potential to induce degradation of articular cartilage[1]. Synovectomy is used to remove the inflamed hypertrophic synovium, and consequently to stop the inflammatory process caused by the underlying disease. Radiosynovectomy provides a simple and cost-effective alternative to surgical synovectomy[2] when repeated injections of cortisone fail to suppress inflammation in rheumatoid arthritis (RA)[3]. Radiosynovectomy is a useful alternative to surgical synovectomy if a patient is unable to have surgery or it is difficult to perform surgery in the joint[4]. For example, hemophilic patients with recurrent hemarthrosis benefit from radiosynovectomy[5,6].

Holmium-166 (^{166}Ho) is a radionuclide with high ß-radiation energy ($ß_{max}$ 1.8 MeV), enabling 90% of the radiation to penetrate 2 mm into the thickened synovium[7]. Although extraarticular leakage of ^{166}Ho-ferric hydroxide macroaggregate (^{166}Ho-FHMA) has been reported 3 days after radiosynovectomy in rabbits[8], this leakage did not result in high radiation exposure to unrelated tissues due the relatively short half-life (27 h) of ^{166}Ho[9,10].

Radiation has been claimed to predispose the treated joint to osteoarthritis (OA)[11]. However, radiosynovectomy with ^{166}Ho-FHMA did not produce permanent deleterious effects on lapine cartilage[12], and no histological damage was observed in normal equine articular cartilage after radiosynovectomy[13]. These studies, however, lack data on the effects of radiosynovectomy on OA cartilage. Radiosynovectomy is performed to treat recurrent synovitis, and the ongoing synovial inflammation promotes degradative changes in articular cartilage[1]. Thus the joint under treatment may show osteoarthritic changes before intraarticular radiopharmaceutical is administered.

In racehorses, traumatic synovitis and deterioration of articular cartilage are common in the dorsal aspect of the metacarpophalangeal (MCP) and the metatarsophalangeal

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Supported by MAP Medical Technologies Oy, Tikkakoski, Finland.

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Submitted February 11, 2003; revision accepted July 8, 2003.

(MTP) joints[14]. Degeneration of articular cartilage near the joint margin is thought to begin early in a horse's life due to the inflammatory mediators released by trauma from the joint capsule following repetitive overextension of the joint[14]. The articular cartilage located within a range of 1 to 2 cm from the dorsal articular margin shows mild deterioration in all racehorses secondary to traumatic synovitis or capsulitis[14]. Severe trauma causes erosion of the articular cartilage, radiographic changes, and clinical signs of joint illness[14-16]. We investigated the effects of ^{166}Ho-FHMA radiosynovectomy on adult equine articular cartilage by measuring cartilage compressive stiffness in vivo with an arthroscopic indentation device applicable to human and equine joints[17,18]. The results were complemented with morphometric, biochemical, and molecular biologic analyses of the cartilage.

MATERIALS AND METHODS

Animals. Six adult mixed-breed horses (all mares, mean age 9 yrs, range 6–14 yrs, mean weight 475 kg, range 444–495 kg), with no signs of OA on physical examination and normal morphology based on radiographs of MCP and MTP joints, were selected for the study. During the experiment the horses were kept either in standard box stalls or in paddocks. Horses were monitored daily for signs of lameness and discomfort during the experiment, which was approved by the Animal Care and Use Committee of the University of Helsinki. Data for one control MTP joint were discarded from the study due to a wound infection after arthroscopy requiring intraarticular treatment.

^{166}Ho-FHMA injections and joint fluid collections. ^{166}Ho-FHMA was injected into the MCP and MTP joints (fetlock joints) under aseptic conditions through the lateral collateral sesamoidean ligament. One joint was injected with 5 ml radioactive ^{166}Ho-FHMA (the ^{166}Ho-FHMA treated joint) and the contralateral joint with 5 ml nonradioactive ^{165}Ho-FHMA (the control joint). All injections were made after horses were sedated with detomidine (10–20 µg/kg bwt intravenously; Domosedan®, Orion, Espoo, Finland). The mean ^{166}Ho-FHMA dose in the radiosynovectomized joints of 1000 MBq (range 688–1154 MBq) was extrapolated from a preliminary study on humans[10]. Injected activity was determined by assaying the amount of activity in the syringe before and after injection in a well counter (Isaocal II, Vinten, UK). ^{166}Ho-FHMA was prepared by MAP Medical Technologies Oy, Tikkakoski, Finland. Injections into the MTP joints were performed at 5 days and into MCP joints at 2 months before the animals were sacrificed. Joint fluid was first collected at the time of ^{166}Ho-FHMA treatment, and repeated immediately before the horses were killed for acquisition of cartilage samples.

Joint fluid analyses. After centrifugation of the synovial fluid samples the supernatant was frozen at –80°C and subsequently assayed for sulfated glycosaminoglycans (GAG) after digestion with papain (Sigma, St. Louis, MO, USA) for 1–2 h at 60°C. GAG were measured by the 1,9-dimethylmethylene blue binding assay[19].

Arthroscopic examinations. Arthroscopic stiffness measurements were performed on the dorsal aspect of the fetlock joints 5 weeks before the ^{166}Ho-FHMA injections and were repeated immediately before the horses were killed. Stiffness data were obtained for the condylar surface and the sagittal ridge on the medial side of the dorsal joint area (Figure 1). Compressive testing of articular cartilage was performed with an arthroscopic indentation instrument validated for stiffness measurements of thin articular cartilage (Artscan 1000, Artscan Oy, Helsinki, Finland)[20]. The contact plate was modified, i.e., elevated and reduced in size to enable measurements of small cartilage areas using a pressing force of 7 N.

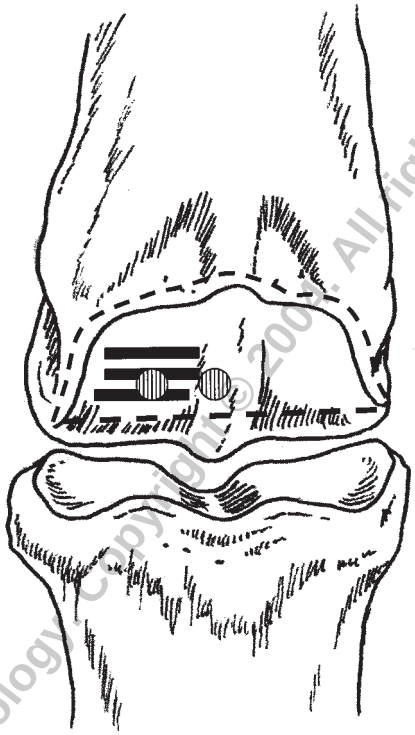

Figure 1. Sampling sites used in the equine fetlock joint. Two cartilage areas (spots with stripes) were used for biomechanical analyses. Three slices (black bars) of 2 mm were cut from the medial condylar surface of metatarsal III and metacarpal III bones for morphometric and biochemical studies. Cartilage from the dorsal condylar surface (area inside the broken line) was taken for RNA extraction and Northern analysis.

Preparation of cartilage samples. After the intact fetlock joints were removed at necropsy, the joints were opened and 3 cartilage slices were cut from each specimen site (Figure 1) with a high-speed drill equipped with 2 parallel cutting disks and a spacer between. Constant flushing with ice-cold 0.9% sodium chloride was used to prevent heating of the samples. The first slice was taken from the medial condylar surface 2 cm from the dorsal articular margin for histology, quantitative autoradiography, and densitometric analysis of GAG. The second slice, 2 mm dorsal to the first, was prepared for electrophoretic analysis of proteoglycans; and the third from the dorsal joint area close to the articular margin for analysis of total sulfate incorporation and uronic acid concentrations. These slices were transferred into ice-cold minimum essential medium (MEM) supplemented with Earle's salts (Life Technologies, Paisley, Scotland) and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). The tissue pieces were preincubated at 4°C until the next day, when they were labeled with ^{35}S-sulfate (50 µCi/ml) in Dulbecco's MEM at 37°C in a 5% CO_2 atmosphere. After 6 h, the labeling medium was removed and the samples were washed 3 times with ice-cold phosphate buffered saline.

Cartilage histology. Specimens for histology were fixed in 4% formaldehyde at 4°C for 20 h followed by decalcification in buffered 10% EDTA for 12 days at room temperature, dehydration with alcohol, and embedding in Paraplast Plus® wax (Sherwood Medical, Kildare, Ireland). Specimens were cut into 3 µm sections for safranin-O staining, and into 5 µm sections for hematoxylin-eosin (H&E) staining. Degenerative changes of articular cartilage were blindly evaluated by the first author in the H&E stained sections according to the following scale: 0 = normal cartilage, 1 = surface frayed (fibrillation), 2 = horizontal fibrillation, 3 = vertical fibrillation, and 4 = erosion of cartilage[21].

Autoradiography. For autoradiography, tissue sections were prepared as described[22,23], with the exception that no background staining was used. Microscopic analysis of grain area fraction, giving an unbiased estimate of grain volume fractions in the sections, was performed using image analysis[22]. Each microscopic view was divided into 10 fields from cartilage surface to the tidemark. The 2 most superficial fractions represented the superficial zone, the 4 consecutive fields the middle zone, and the deepest 4 the deep zone. The results are presented as the average grain fraction percentage of the total tissue volume.

Digital densitometry of GAG and thickness measurement of uncalcified cartilage. Analysis of GAG concentration in uncalcified cartilage was carried out as described using safranin-O stained sections[24,25]. The measured area spanned from the cartilage surface down to the tidemark. The total thickness of uncalcified cartilage was recorded and each full-depth cartilage area measured was divided into 12 fractions, each corresponding to 1/12 of the total thickness and a constant width of 620 µm. The 2 most superficial fractions were considered to represent the superficial zone, the next 5 fractions the middle zone, and the deepest 5 fractions the deep zone of articular cartilage. The results were calculated as mean area-integrated optical density proportional to 1 μm^2 in each zone (OD/μm^2).

Sodium dodecyl sulfate (SDS) agarose gel electrophoresis of the proteoglycans. A surgical knife was used to release cartilage from the subchondral bone. After determination of wet weight, cartilage was sliced into small pieces and proteoglycans were extracted with 500 µl of 4 M guanidinium hydrochloride in 50 mM sodium acetate (pH 5.8) containing inhibitors of bacterial growth and proteases[23]. Extraction was continued at 4°C for 24 h. The extracts were then precipitated in 75% ethanol at 4°C and washed twice in ice-cold 70% ethanol. Uronic acid was measured with the m-hydroxydiphenyl method[26]. The samples were dissolved in SDS and electrophoresed in submerged horizontal 5 mm-thick 1.2% agarose gels[27]. The gels were stained with toluidine blue, dried, and scanned for densitometry, and finally exposed to x-ray films for 2 weeks. The scanned film and gel densities were analyzed with image analysis software (IP-Lab, Scananalytics, Fairfax, VA, USA)[23].

Total incorporation of ^{35}S-sulfate. After in vitro labeling, cartilage was dissected free from underlying bone, weighed, and digested overnight at 60°C with 0.05% proteinase K (Roche Boehringer Mannheim, Mannheim, Germany) and 10 mM EDTA. Aliquots of digests were eluted through Sephadex G-25 gel filtration columns (PD-10, Amersham Pharmacia-Biotech, Uppsala, Sweden) to remove unincorporated ^{35}S-sulfate. The radioactivity in each sample was determined by liquid scintillation counting (Winspectral™, Wallac, Turku, Finland) of PD-10 eluates and of medium samples using a water-soluble scintillation cocktail (Optiphase Hisafe III, Wallac).

Northern analysis. For extraction of total RNA, a razor blade was used for meticulous removal of articular cartilage from the dorsal aspect of the distal metacarpal and metatarsal bones (Figure 1). Tissue slices were frozen, powdered under liquid nitrogen, homogenized in 4 M guanidinium isothiocyanate, and sedimented through 5.7 M cesium chloride as described[28,29]. For Northern analyses, 10 µg aliquots of total RNA were denatured with glyoxal and DMSO, electrophoresed onto 0.75% agarose gels, and transferred overnight with 20× SCC onto Pall Biodyne membranes for hybridizations with ^{32}P-labeled probes under standard conditions as recommended by the supplier (Pall Europe, Portsmouth, UK). The bound probes were detected and quantified on a molecular phosphoimager (Bio-Rad, Hercules, CA, USA) and the signals were corrected for variations in the 28S rRNA levels determined by hybridization.

Hybridization probes. The mRNA for aggrecan core protein and pro-α_1(II) collagen was detected using cDNA clones pHAgg[30] and pECol2a1-1, respectively. The cDNA probe for the detection of equine pro-α_1(II) collagen was constructed using the reverse transcription-polymerase chain reaction (RT-PCR) technique with equine articular cartilage RNA as template. Oligo$(dT)_{18}$ and random hexamers were used as primers for the first-strand cDNA synthesis, and 2 specific oligonucleotides based on the published equine sequence[31] as amplification primers. The PCR reaction was carried out in standard conditions, and the product was cloned into a pGEM T plasmid vector (Promega, Madison, WI, USA) and sequenced completely to verify its identity. Clone pECol2a1-1 spans 211 nucleotides and covers nucleotides 4361–4571 of the published sequence[31]. For the detection of 28S rRNA levels, cDNA clone 341-1 was used[32]. For Northern hybridizations, the inserts were purified from each plasmid after appropriate restriction digest and radiolabeled with ^{32}P-dCTP using the random priming method (Boehringer Mannheim).

Statistical analysis. Differences in measured variables between treated and corresponding control fetlock joints were tested with the nonparametric Wilcoxon matched-pairs signed-rank test, since the data did not fulfill the criteria for parametric testing.

RESULTS

Equine fetlock joints are well suited for repeated in vivo measurements of the compressive stiffness of articular cartilage. The small size of the contact plate in the indentation instrument enabled analysis of the slightly convex articular cartilage surface (Figure 2). The initial measurements revealed that the cartilage was stiffer on the sagittal ridge than on the condyle. No differences were observed between the reference and followup measurements 5 days after the radiosynovectomy. However, 2 months after radiosynovectomy articular cartilage on the sagittal ridge, but not on the condylar surface, was softer in the treated joint than in the control joint (Table 1).

Despite visually normal cartilage, various histological signs of degeneration were present in both the radiosynovectomized and control joints, e.g., disruption of the superficial cartilage layers and the appearance of horizontal and vertical clefts in the intermediate layer (Figure 3). Of note, radiosynovectomy with ^{166}Ho-FHMA did not aggravate

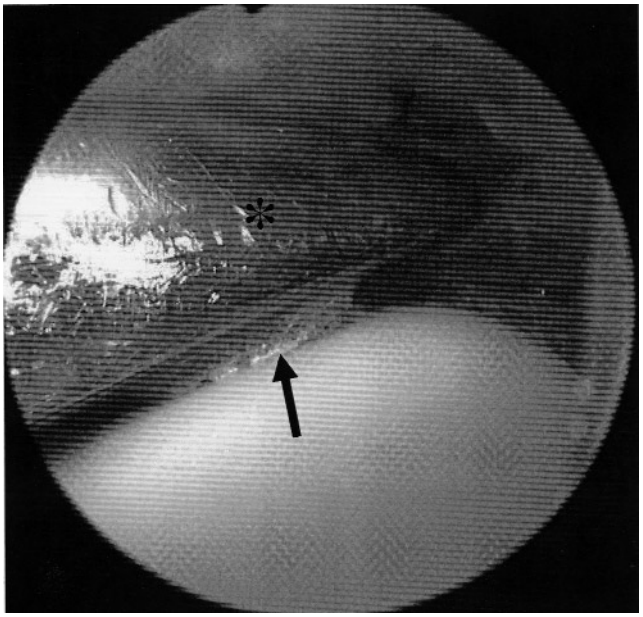

Figure 2. Indentation analysis of the sagittal ridge (to the right) with the arthroscopic instrument (*) with an elevated reference plate (arrow). This is an arthroscopic view.

Table 1. Stiffness of articular cartilage of the condylar surface and the sagittal ridge on the dorsal aspect of the distal metatarsus and the distal metacarpus measured before (Reference) and after (Followup) treatment with ^{166}Ho-FHMA. Stiffness is indicated by the indenter force (N)[17]. The number of paired analyses is shown in parentheses.

Site of Analysis	Condyle		Transverse Ridge	
	Reference	Followup	Reference	Followup
Distal metatarsus 5 days after radiosynovectomy				
Synovectomy (6)	2.05 ± 0.21	1.80 ± 0.13	2.44 ± 0.11	2.24 ± 0.33
Control (5)	1.71 ± 0.13	1.66 ± 0.13	2.31 ± 0.29	2.04 ± 0.15
Distal metacarpus 2 months after radiosynovectomy				
Synovectomy (6)	1.77 ± 0.15	1.75 ± 0.14	2.83 ± 0.16	1.72 ± 0.30*
Control (6)	1.54 ± 0.28	1.96 ± 0.11	2.62 ± 0.22	2.10 ± 0.07

Data represent the mean ± SEM. * Analysis by 2 tailed nonparametric Wilcoxon matched-pairs signed-ranks test, $p < 0.05$.

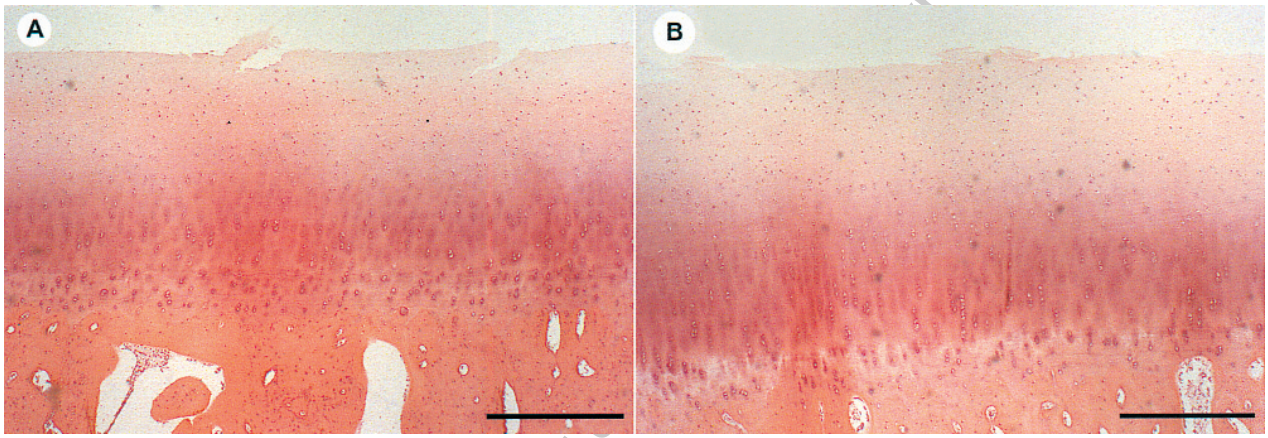

Figure 3. Histology of articular cartilage of the distal metacarpus showing disruption of superficial cartilage surface and clefts into the intermediate zone in ^{166}Ho-FHMA treated joints (A) and control joints (B). H&E staining. Bar = 500 µm.

these degenerative changes. The mean score for cartilage degeneration in the control group was 2 (range 1–3) and in the ^{166}Ho-FHMA treated group it was 1.8 (range 1–3). The thickness of the uncalcified cartilage was equal in the radiosynovectomized and control joints. In the MTP joints the mean cartilage thickness was 633 µm and in the MCP joints 729 µm.

Although a slightly increased GAG content was observed in the synovial fluid from radiosynovectomized joints, this increase did not reach statistical significance (Table 2). Synovectomy with ^{166}Ho-FHMA did not alter uronic acid content in the cartilage samples (Table 2), and densitometric analysis of GAG in safranin-O stained sections showed no zonal differences in the GAG concentration of the articular cartilage between radiosynovectomized and control joints (data not shown). Electrophoretic analysis of the extracted proteoglycans showed a slightly decreased size of the resident proteoglycan monomers in the radiosynovectomized MCP joints (Figure 4).

Two months after radiosynovectomy, the incorporation rate of ^{35}S-sulfate decreased from 5.0 ± 1.1 pmol/mg wet weight/h (mean ± SEM) in the control joints to 1.8 ± 0.8 pmol/mg wet weight/h in the radiosynovectomized joints. The change was not statistically significant. Autoradiographic analysis of cartilage zones after the ^{166}Ho-FHMA radiosynovectomy (Figure 5) indicated that chondrocytes actively synthesized proteoglycans in all of the zones, and no indication of increased cell death was seen.

Table 2. Glycosaminoglycan content of synovial fluid (µg GAG/ml) and articular cartilage (µg/ uronic acid/mg wet weight) in ^{166}Ho-FHMA treated and control joints. Number of paired analyses is shown in parentheses.

	At 5 Days	At 2 Months
Synovial fluid		
Synovectomy	68.4 ± 5.7 (11)	66.4 ± 6.7 (6)
Control	58.6 ± 5.9 (11)	55.8 ± 3.5 (6)
Articular cartilage		
Synovectomy	5.1 ± 0.7 (5)	6.4 ± 1.1 (6)
Control	5.8 ± 0.3 (5)	5.9 ± 0.5 (6)

The values are expressed as mean ± SEM. The 2 tailed nonparametric Wilcoxon matched-pairs signed-ranks test was used for statistical analysis; no significant differences were found between the synovectomy and control groups.

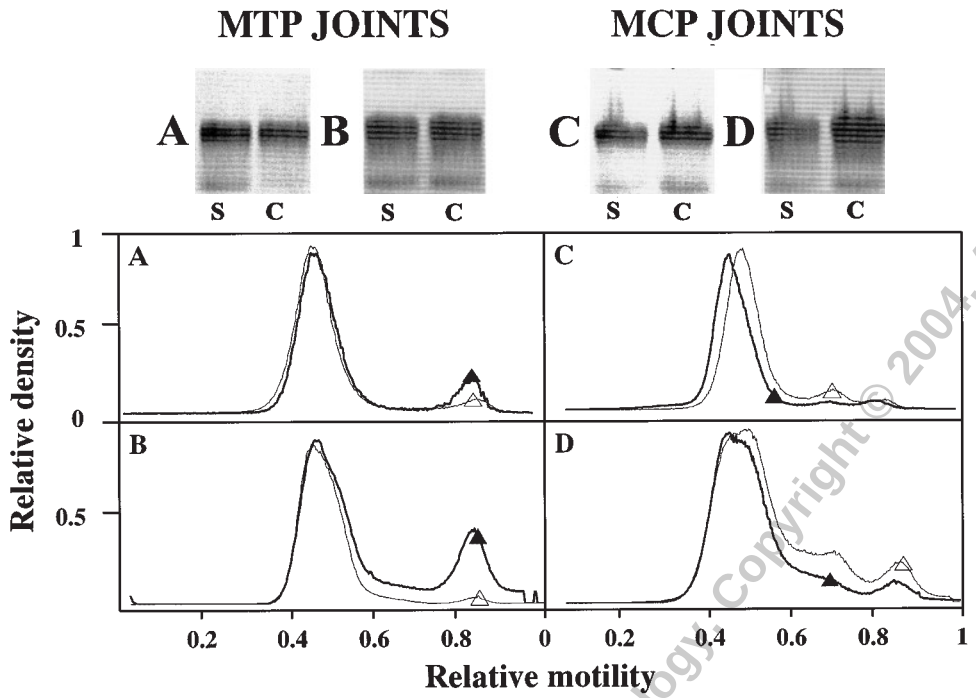

Figure 4. SDS agarose gel electrophoresis of proteoglycans from equine cartilage. Proteoglycans were extracted from cartilage samples from the dorsal condylar surface at 5 days (MTP joints) and 2 months (MCP joints) after ^{166}Ho-FHMA treatment. Curves show densitometric analysis of autoradiography films (A, C) and stained gels (B, D). Respective films and gels are shown at top. S: radiosynovectomized (△); C: contralateral control (▲) sample.

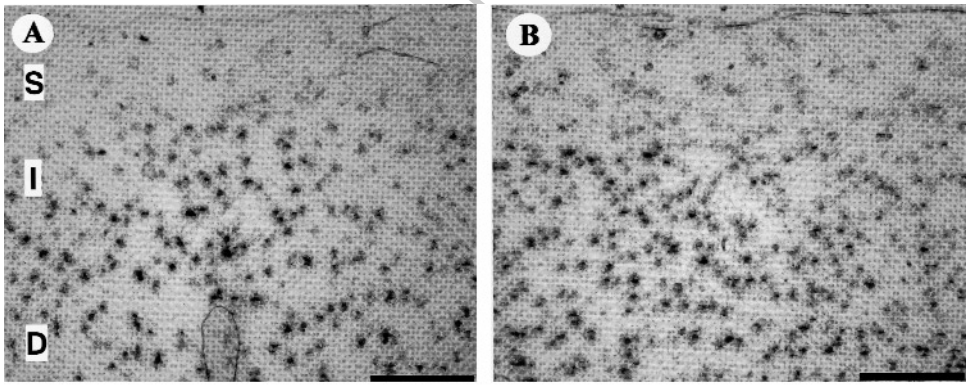

Figure 5. Autoradiography of sections of articular cartilage at the condylar surface. Two months after ^{166}Ho-FHMA treatment, equal levels of ^{35}S-sulfate incorporation were seen in ^{166}Ho-FHMA treated samples (A) and control samples (B). S: superficial zone; I: intermediate zone; D: deep zone. Bar = 500 µm.

The levels of pro-α_1(II) collagen were slightly reduced in the ^{166}Ho-FHMA treated joints 5 days after radiosynovectomy compared with the contralateral control joints, whereas aggrecan mRNA levels remained unchanged. At 2 month followup, no difference was observed in mRNA levels between the ^{166}Ho-FHMA treated and control joints (Figures 6 and 7).

DISCUSSION

This study demonstrates that radiosynovectomy using a single high intraarticular dose of ^{166}Ho-FHMA (mean 1000 MBq/joint) does not aggravate histological changes in naturally degenerated adult articular cartilage near the dorsal joint margin of the fetlock joint in racehorses. However, stiffness measurement with the indentation device indicated slightly softer articular cartilage on the sagittal ridge 2 months after radiosynovectomy. Softening of articular cartilage could be associated with a slight loss of proteoglycans from the cartilage and reduction of proteoglycan synthesis by the chondrocytes[33]. However, biochemical analyses did

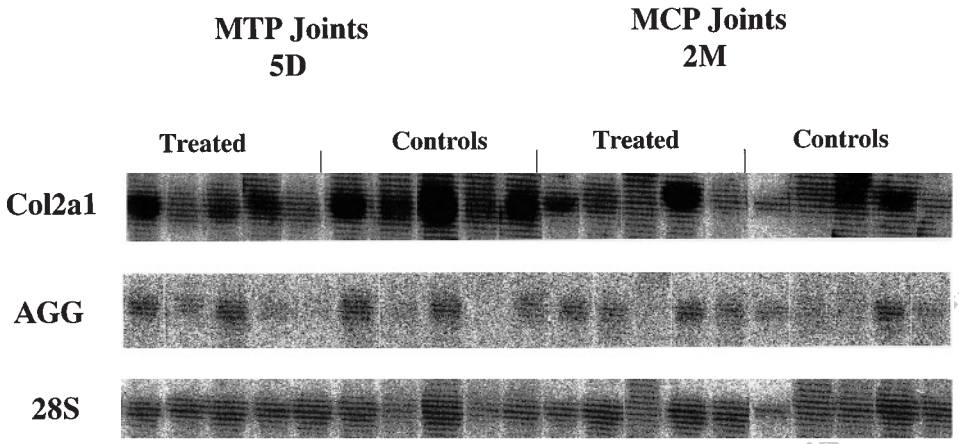

Figure 6. Expression of pro-α_1(II) collagen and aggrecan mRNA in articular cartilage. Hybridization signals were quantified by phosphorimaging, and the values were normalized against a 28S rRNA signal. Col2a1: pro-α_1(II) collagen; AGG: aggrecan; 5D: 5 days after treatment; 2M: 2 months after treatment.

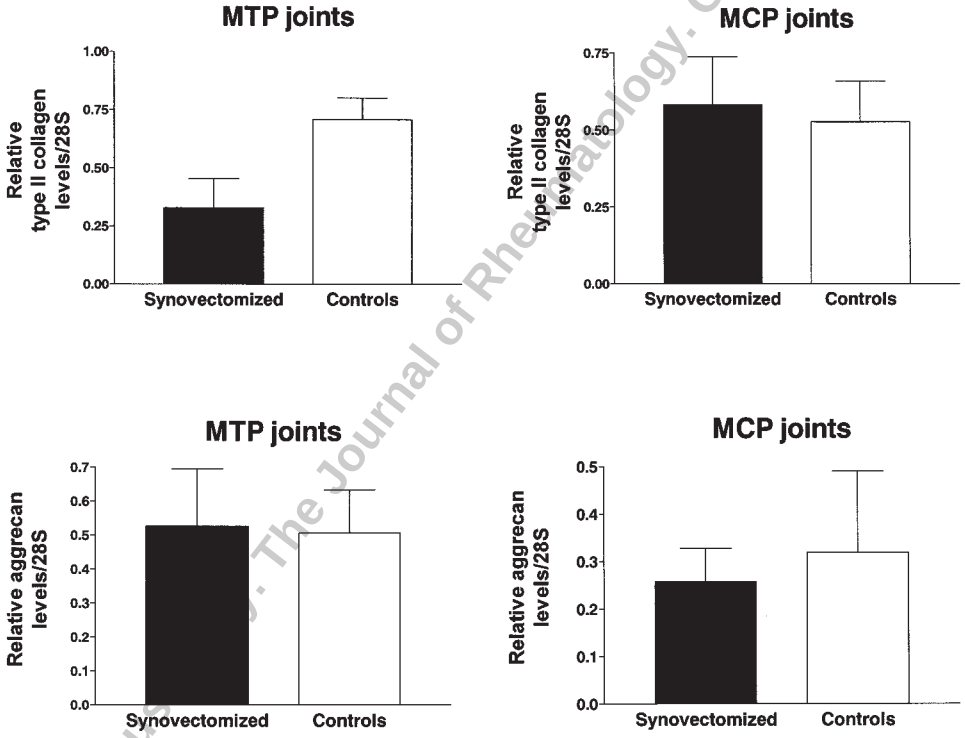

Figure 7. A summary of changes in mRNA levels of pro-α_1(II) collagen and aggrecan after ^{166}Ho-FHMA treatment. Data compiled from quantitative analyses of Northern hybridizations from MTP (5 days post-^{166}Ho-FHMA) and MCP joints (2 months post-^{166}Ho-FHMA).

not confirm loss of proteoglycans from the cartilage. Further, it was impossible to measure exactly the same spots at various times with the small contact plate. Therefore, natural degeneration probably explains the softening of the cartilage on the sagittal ridge. The naturally-occurring cartilage degeneration in this study differs from the experimental antigen-induced arthritis (AIA) model often used to simulate human RA[34-37]. In AIA the cartilage lesions differ from those of true OA and the mechanisms of cartilage breakdown are also not comparable to OA[38,39].

Loss of proteoglycans (PG) is one of the major events in OA and thus we analyzed the content of GAG in articular cartilage and in joint fluid of control and radiosynovectomized joints. Changes in the quantity, quality, and organization of proteoglycans have been associated with OA, even in the absence of major histological alterations[40,41]. The

slight increase in joint fluid GAG concentration suggested increased turnover and/or release of PG after radiosynovectomy, but the biochemical analyses did not confirm any marked change in the composition of the equine cartilage during the 2 month followup. The reduced size of articular cartilage PG 2 months after ^{166}Ho-FHMA treatment possibly reflected an early stage of the degeneration process[42]. However, it is uncertain whether small size of PG monomers is always associated with OA[43].

Radiosynovectomy has been suggested to change the metabolic activity of chondrocytes and, in the worst case, even to result in chondrocyte death at the articular surface[34,44]. In dogs, radiosynovectomy with a strong ß-emitter, yttrium-90, was shown to increase the synthesis of PG[45], which has been associated with cartilage damage[46]. In contrast, in our study with ^{166}Ho-FHMA radiosynovectomy, PG synthesis was decreased rather than increased. However, the autoradiographic analysis did not support the observation of marked cell death caused by the isotope, and neither was any corresponding decrease observed in aggrecan mRNA levels after the ^{166}Ho-FHMA treatment. Since other studies have shown a rather consistent relationship between aggrecan mRNA levels and PG production in articular cartilage chondrocytes[47], the discrepancy in our results could possibly be explained if the effect of ^{166}Ho-FHMA treatment on chondrocytes was strongest close to the joint margin.

The levels of mRNA for cartilage matrix components, especially type II collagen, are high in growing and immature cartilage, but very low in mature cartilage[48]. Northern analysis has shown increased levels of mRNA for pro-α_1(II) collagen in OA cartilage, suggesting reactivation of type II collagen synthesis[31,49]. In our study, no radiosynovectomy-related increase was seen in mRNA levels for pro-α_1(II) collagen or aggrecan. The slightly lowered mRNA level for pro-α_1(II) collagen detected 5 days after radiosynovectomy could be the result of transient cytokine-induced downregulation associated with radiation-induced joint inflammation[31,50].

In summary, the stage of cartilage degeneration, cartilage thickness, or composition did not change due to radiosynovectomy; only minor degradation of PG monomers was observed. In other studies, chondrocyte damage, cartilage degeneration, and multifocal necrosis of synovium have been reported[8,11,34,44,51]. Increased metabolic activity of chondrocytes may protect cartilage from damage in immature animals[12], but chondrocytes in mature cartilage show a much lower level of matrix production even if slightly activated in OA[48]. However, ^{166}Ho-FHMA treatment did not lead to increased mRNA levels for pro-α_1(II) collagen or aggrecan despite minor alterations of chondrocyte metabolism. Increased concentrations of inflammatory mediators and degradative enzymes are found in the synovial fluid of horses in naturally-occurring synovitis[52,53]. Joints with chronic proliferative synovitis could therefore benefit from ^{166}Ho-FHMA treatment through suppression of the ongoing inflammation. Preservation of cartilage after treatment of proliferative synovitis with ^{166}Ho-FHMA should be evaluated in a further study.

ACKNOWLEDGMENT

The authors thank Eija Rahunen, Elma Sorsa (Department of Anatomy, University of Kuopio) and Merja Lakkisto (Department of Medical Biochemistry and Molecular Biology, University of Turku) for excellent technical assistance. We also thank Pirkko Penttilä, MS (MAP Medical Technologies Oy, Tikkakoski, Finland) for preparing radiopharmaceutical ^{166}Ho-FHMA.

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