Osteoarthritis and Cartilage (2001) **9,** 371–381
© 2001 OsteoArthritis Research Society International
doi:10.1053/joca.2000.0398, available online at http://www.idealibrary.com on IDEAL®
1063–4584/01/040371+11 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Morphological analysis of articular cartilage biopsies from a randomized, clinical study comparing the effects of 500–730 kDa sodium hyaluronate (Hyalgan®) and methylprednisolone acetate on primary osteoarthritis of the knee

D. D. Guidolin*, I. Pasquali Ronchetti†, E. Lini*, D. Guerra† and L. Frizziero‡
**FIDIA Research Laboratories, Abano Terme, Italy*
†*Biomedical Sciences Department, University of Modena and Reggio Emilia, Italy*
‡*Department of Internal Medicine, Rheumatology Unit, Ospedale Maggiore, Bologna, Italy*

Summary

Objective: Histomorphometric study on cartilage samples taken from osteoarthritic human knees before and 6 months after intraarticular injections of a specific fraction (500–730 kDa) of hyaluronan. The results obtained with hyaluronan were compared with the results of methylprednisolone acetate treatment.

Methods: Twenty-four subjects with primary osteoarthritis (OA) of the knee were considered. Eleven patients were treated with Hyaluronan (Hyalgan®, 20 mg/2 ml once a week for 5 weeks) and 13 with methylprednisolone (Depo-Medrol®, 40 mg/1 ml once a week for 3 weeks). At the time of baseline and after 6 months from the start of treatment, biopsies of cartilage were taken and processed for electron microscopy. Articular surface morphology, territorial matrix, chondrocyte number and ultrastructure were characterized by a set of morphometric parameters. Samples from 19 informed patients showing no arthroscopic sign of OA were also used for comparison.

Results: Six months after hyaluronan treatment a significant reconstitution of the superficial layer were observed together with an improvement in chondrocyte density and territorial matrix appearance. Furthermore, chondrocytes appeared significantly improved in their metabolism, as indicated by the increased extension of the synthetic structures and mitochondria with respect to the organelles having catabolic or storage functions. Hyaluronan treatment produced results that were significantly superior to those delivered with Methylprednisolone in almost all the morphometric estimators.

Conclusions: These results cannot be explained simply by temporary restoration of the synovial fluid viscoelasticity, and provide further evidence that the specific fraction of hyaluronan used in this study is a useful tool in OA treatment, with a potential structure-modifying activity. © 2001 OsteoArthritis Research Society International

Key words: Hyaluronan, Osteoarthritis, Cartilage, Morphometry.

Introduction

Osteoarthritis (OA) is presently regarded as a disease process affecting the entire joint[1], originated by a variety of mechanisms and resulting in a progressive degradation of the articular cartilage. Current therapy for OA is capable of alleviating the symptoms but no agent has, as yet, been definitively shown to slow down the progression of cartilage damage. Significant resources are now being dedicated to the search for drugs characterized by structure-modifying activity, i.e. able to slow down the rate of cartilage degeneration and/or enhance that of cartilage repair[2,3].

Hyaluronic acid (HA) is a natural molecule playing a pivotal role in all the regions of the joint. It is actively synthesized by the synoviocytes and it determines the viscoelastic properties of the synovial fluid. In the cartilage, permanently complexed with proteoglycans, HA is essential in mantaining the structural and functional characteristics of this tissue. In addition, HA, either free or weakly associated with short proteoglycan structures, contributes to the formation of an amorphous layer about 0.6 μm thick, covering the articular surface of the cartilage[4], contributing to the boundary lubrication mechanism in conditions of extreme loading[5] and partially protecting the tissue from the penetration of inflammatory cells and lytic enzymes.

It is well known that in pathological processes, such as OA and rheumatoid arthritis (RA) the molecular weight of the HA in the synovial fluid can be reduced by an order of magnitude (from 10^6 to 10^5 Da) and its concentration is also reduced mainly due to the accumulation in the joint cavity of liquid derived from the inflamed synovial vessels[6,7]. The result is a reduction in the viscoelasticity of the fluid and an increased susceptibility of cartilage to breakdown.

To counteract this process a therapeutic strategy based on the use of intraarticular injections of exogenous HA started in the late 1960s[8,9]. Since then a large number of studies has been published demonstrating the positive and

Received 20 December 1999; revision requested 12 April 2000; revision received 26 September 2000; accepted 7 November 2000.
This study was supported by a grant from FIDIA S.p.A.
Address correspondence to: Dr D. Guidolin, Fidia Research Laboratories, Via Ponte della Fabbrica, 3A, 35031 Abano Terme, Italy. Tel: +39-49-8232330; Fax: +39-49-8232120. E-mail: dguidolin@fidiapharma.it

long lasting effects of HA treatment in human and animal joint diseases[10–20].

Very recently, the effects of HA therapy on human joint tissues have been directly monitored using arthroscopy and histomorphometric analysis of biopsies[21,22]. The results documented that HA treatment induced an improvement of several structural features of both synovial membrane and cartilage, opening the possibility for HA to be a candidate structure (disease-) modifying drug.

In order to further study the effects of a specific fraction of HA (500–730 kDa) on the structure of joint tissues in OA, we carried out a histological and electron microscopy study on cartilage samples taken from osteoarthritic human knees before and 6 months after local injections of Hyalgan® (FIDIA SpA, Italy). The results obtained with Hyalgan were compared with the results of methylprednisolone acetate (Depo-Medrol®) treatment.

Materials and methods

STUDY DESIGN

The study is focused on biopsy samples coming from a clinical trial carried out on 50 patients, aged from 38 to 73 years, with primary OA of the knee. The trial was a 6-month, randomized, controlled study, in which OA was diagnosed following the criteria of the American College of Rheumatology[23] and was arthroscopically confirmed.

Patients judged uncontrollable or unreliable, those with severe concomitant diseases making assessment of the result difficult, those receiving therapies during the previous 3 months (apart from oral administration of non-steroid antiinflammatory drugs) and women in pregnancy or breast feeding were excluded from the trial. Consecutive outpatients with clinically and radiologically diagnosed unilateral or bilateral OA of the knee (Kellgren–Lawrence grades I–III) were admitted to the study. The rate of acceptance to enter the study was 60%. Patients judged eligible underwent a first knee arthroscopy during which biopsies of cartilage were obtained.

Half of the recruited patients were to be treated with hyaluronan (Hyalgan®, FIDIA SpA) and half with methylprednisolone (Depo-Medrol®, Pharmacia & Upjohn). Patients were recruited into one of the two treatment groups following a randomization scheme generated by computer. In presence of bilateral OA, the more severely affected knee was selected for treatment.

Within 15–20 days of the arthroscopic inspection, all these patients had a baseline visit with collection of blood and urine samples for routine laboratory assessments. They then started on a course of intraarticular injections of 2 ml Hyalgan® (10 mg/ml 500–730,000 MW hyaluronan in saline) once a week for 5 weeks, or 1 ml Depo-Medrol® (40 mg/ml 6-methylprednisolone acetate in saline) once a week for 3 weeks.

On days 7, 14, 21, 28, 35, 60, 120 and 180 clinical parameters were assessed and blood and urine samples collected for laboratory analysis. During this period no patient of either group took analgesic drugs or used NSAIDs regularly. On day 180 each patient underwent a second arthroscopic examination and, if the patient agreed, a second biopsy for morphological analysis was performed. Only cartilage samples coming from patients with primary OA, who underwent both basal and final cartilage sampling (Table I) were considered for the present study.

Table I
Profile of patients

	Hyaluronan	Methylprednisolone
No. of subjects	11	13
Male	4	7
Female	7	6
Age (mean±S.E.M.)	51.5±2.8	57.0±1.7

A normal control group of cartilage samples was also obtained from 19 subjects who underwent arthroscopy for pain, but did not reveal any sign of either OA or RA.

The trial was approved by the Ethical Committee of the Ospedale Maggiore of Bologna. Written informed consent for treatment, arthroscopic inspections and sampling was obtained from all patients.

ARTHROSCOPIC EXAMINATION AND BIOPSY SAMPLING

Microarthroscopy was always performed by the same investigator, using Hamou-Storz and Microview-Wolf arthroscopes equipped with a ×1 to ×150 selection of magnification and automatic focus control. Local anesthesia was induced by injections of 2 ml 2% Mepivacaine-HCl without a vasoconstrictor. The endoscope was introduced antero-laterally keeping the knee flexed at an angle of 30°. Intermittent irrigation with Ringer's acetate was used to mantain the intraarticular pressure around 80 mm Hg and to optimize the degree of distension of the joint cavity. Three millilitres of 1% methylene blue in aqueous solution (pH 4.5) was injected into the joint cavity, left for 5 min and then exhaustively washed out with saline.

Biopsies of the articular cartilage were only taken from those patients with arthroscopic grade II lesions (i.e. cartilage marked by fibrillation, fissures and a velvety appearance) according to a series of previously described standardized procedures[22]. In each patient the area chosen for sampling was the tissue compartment presenting at baseline the lesion with the greatest superficial extension. Tissue samples were taken from the edge of the lesion, which was easily identified by the more intense staining with methylene blue of the damaged area when compared with healthy tissue.

Because the same tissue region had to be sampled again during the final arthroscopic examination on day 180, to determine the precise sampling area a graded needle-probe was inserted under continuous arthroscopic control, using an independent route of access depending on the tissue compartment involved.

For the medial and lateral femoral condyles, the probe was introduced at the point of intersection of a straight line tangential to the anterior margin of the collateral ligament with the synovio-meniscus junction. It was advanced along the femoral condyle tangent until the apex of the probe coincided with the area chosen for sampling. For the medial and lateral tibial plateau, the route of insertion of the probe was the same as that described for the medial femoral condyle. The probe was advanced along the femoral condyle, tangentially passing the tibial spine until the apex coincided with the area chosen for sampling. For the patella, the probe was inserted medially into the femoropatellar space, advancing along a line coinciding with the median transverse axis of the patella until the apex coincided with the chosen sampling zone. The distribution of

the sampled compartments was comparable in the two treatment groups.

Cartilage specimens of the normal control group were taken from the middle of the edge between the condylar articular cartilage and the intercondylar notch medial surfaces. Biopsies were numbered according to a randomization list, processed and examined without any information apart from the identification number.

SPECIMEN PREPARATION

After excision cartilage samples were immediately dipped in 1% glutaraldehyde (Fluka AG, Buchs, Switzerland) and Tyrode's physiological solution pH 7.2 and then cut under a stereomicroscope in small blocks, less then 0.5 mm wide, taking care to maintain the whole thickness of the cartilage in each fragment. Samples were then fixed by 2.5% glutaraldehyde in Tyrode's pH 7.2, in the presence of 0.1% toluidine blue O (Serva Feinbiochemica, Heidelberg, Germany), for 24 h at 4°C and then by 1% osmium tetroxide (Fluka AG, Buchs, Switzerland) in Tyrode's pH 7.2, containing 0.05% toluidine blue O, for 2 h at room temperature. After dehydration in ethanol and propilene oxide, specimens were embedded in Spurr resin (Polysciences Inc., Warrington, PA), taking care to orient them in order to have on the same section the whole thickness of the cartilage. 0.5 μm-thick sections were stained with 0.1% toluidine blue O in 1% $Na_2B_4O_7$ and used for light microscopy (Phomi III, Carl Zeiss, Oberkochen, Germany), ultrathin sections (70–80 nm thick) were collected on copper grids and stained with uranil acetate and lead citrate for transmission electron microscopy (CM12, Philips, Eindhoven, The Netherlands).

MORPHOMETRY AND STATISTICAL ANALYSIS

At least three random blocks per biopsy were considered for the morphometric analysis and analysed under blind conditions. A set of parameters was determined in the tangential layer (or Zone I) and in the intermediate layer (or Zone II) of the articular cartilage[24] by using a computer assisted image analysis system (IBAS, Kontron, Eching, Germany). At the light microscopy level the number of chondrocytes per unit volume and the volume density of the cells were estimated.

The chondrocyte numerical density was obtained according to the disector method, in which, instead of a single sectioning plane, two parallel planes separated by a known distance (3 μm in our study, since we analysed the first and the seventh section of a consecutive series, each section being 0.5 μm in thickness) are used to count the cells[25]. Volume density of the chondrocytes was estimated by planimetry as the ratio between the area occupied by cell profiles and the sampled tissue area. From these basic estimators the mean cell volume was calculated.

A quantitative measurement of the articular surface roughness was also performed at the light microscopy level. Surface roughness is the degree of deviation of the surface profile from an idealized smooth surface and can be evaluated on tissue sections by calculating, from the digitized surface profile, the parameter RMS (root mean square roughness) as indicated by Hacker *et al*.[26].

In order to estimate the numerical density of metabolically active chondrocytes, the percentage of necrotic cell profiles was evaluated directly at the transmission electron microscope by a systematic sampling of both Zone I and Zone II at a magnification of ×3000.

The quantitative characterization of the chondrocyte ultrastructure was performed according to the approach originally proposed by Annefeld[27]. Micrographs of 10 cell profiles per block in both Zone I and Zone II were taken at a magnification of ×17000 and the volume fraction occupied by the components of the chondrocyte anabolic (Rough Endoplasmic Reticulum (RER), Golgi apparatus, Mitochondria) and catabolic systems (lysosomes, fatty acid vacuoles, glycogen, intermediate filaments) was then estimated by point counting[28].

The mean thickness of the superficial amorphous layer of the cartilage was also measured at the electron microscopy level and its compactness assessed for each block by using the following semiquantitative scale (see Fig. 1): 0, absent; 1, fragmented; 2, discontinuous; 3, compact.

An evaluation of the presence of anomalous territorial matrix (see Ghadially[24]) was obtained in Zone I by recording the eventual occurence of this feature on two ultrathin sections, taken at a distance greater than the mean chondrocyte diameter (~12 μm) from each of the considered blocks for a total of at least six observations per biopsy. The frequency of occurrence was then estimated for each subject. The difference between the frequency observed after treatment and that at baseline was used to establish whether each patient showed improvement, worsening or no changes of the parameter.

Following the same approach, the occurrence in the general matrix of atypical collagen fibers (thicker or branched fibrils[24]) was also estimated.

The basal/final sequence and the type of treatment were matched at the end of the study, when data were processed by a computerized program for statistical analysis (SAS Institute, Cary, NC, U.S.A.). Comparisons of quantitative parameters within groups (final vs basal) were performed by non-parametric tests (Sign Rank Test), while the ANOVA was used to assess differences between treatments. Fisher's exact test was used for the analysis of data concerning the territorial matrix and the collagen fibers. Given the relatively low number of subjects who consented to both biopsies, in order to provide the adequate power to the statistical tests $\alpha \leq 0.10$ was considered statistically significant[29].

Results

BASELINE OBSERVATIONS

The structural appearance of articular cartilage tissue in semithin sections cut perpendicular to the joint surface is illustrated in Fig. 2(b). The articular surface showed fibrillations and fissures and the cell density appeared to be decreased in both zone I and zone II with respect to a normal control sample [Fig. 2(a)].

When observed at the electron microscopy level, all the baseline samples from this study presented detectable modifications of the superficial amorphous layer which covers the free articular surface. The modifications concerned both the thickness and the compactness of this layer (which appeared discontinuous or fragmented into granules). In about 30% of the biopsies taken at baseline it was completely absent.

The presence in zone I of anomalous territorial matrix was observed in more than 70% of the baseline biopsies: a number of chondrocyte profiles appeared lying in unusually

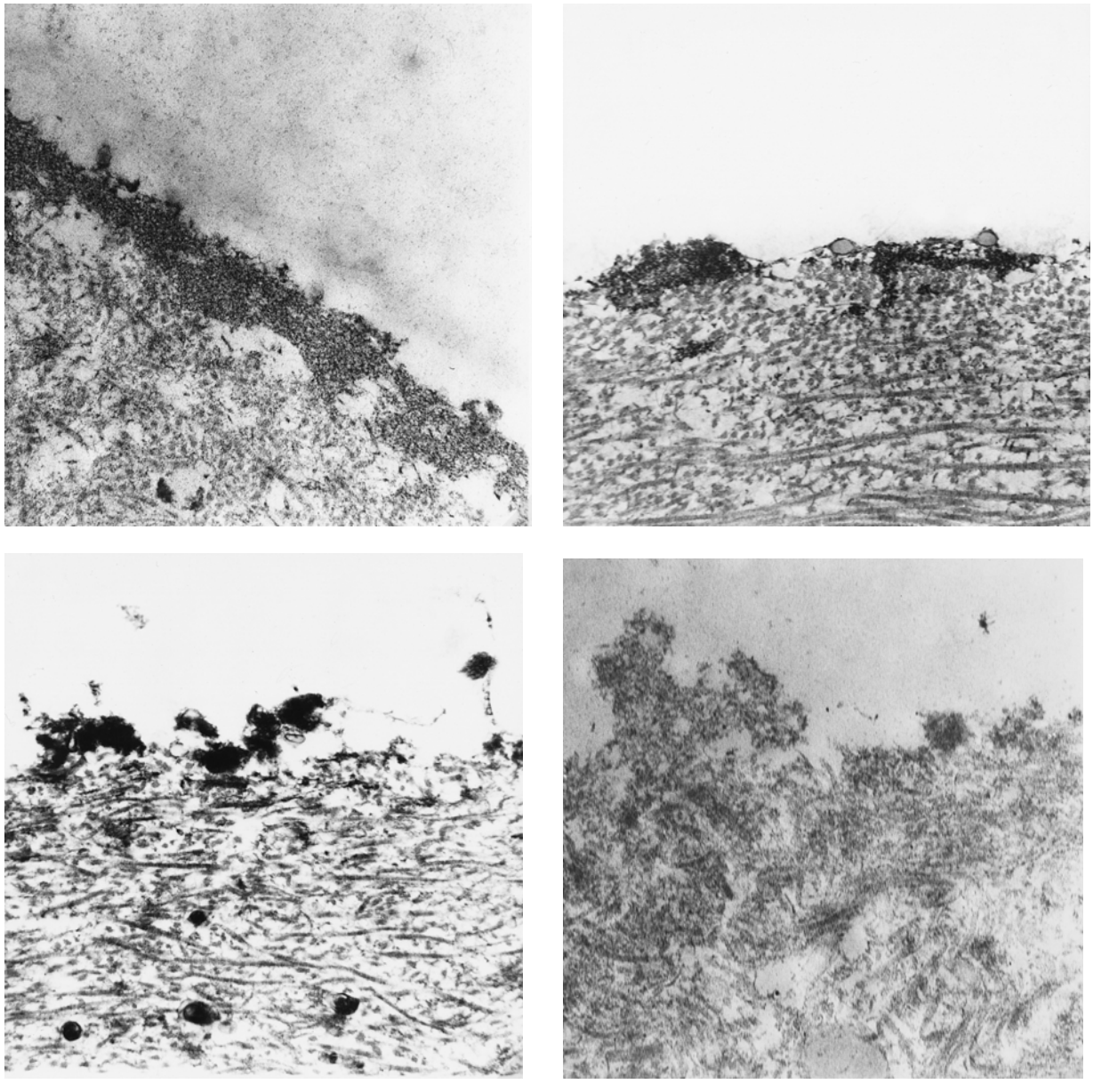

Fig. 1. A series of electron micrographs (primary magnification: ×28000) illustrating different degrees of damage of the superficial amorphous layer of the cartilage. The normal compact appearance is shown in (A). In (B) it exhibits discontinuities and in (C) it appears fragmented in granules. A complete absence of the structure is illustrated in (D).

large amounts of territorial matrix, which itself was atypical (compared with normal samples) in that it was packed with fine collagen fibrils.

In about 90% of the baseline samples atypical collagen fibers were observed in the general matrix. They appeared as short lengths of thicker (~250 nm) fibrils wich stained much more intensely than native collagen fibrils. Collagen fibers with a branching appearance were also frequently detected.

The qualitative observations were confirmed by the morphometric evaluations (Table II) and no significant differences were detected at baseline between the treatment groups.

The articular surface roughness, as measured by the RMS parameter, increased in OA samples by a factor of ~4 (P<0.01) with respect to the value observed in normal tissue samples. Furthermore, the superficial amorphous layer showed a strong decrease of the compactness score and a reduction of the mean thickness of about 60% (P<0.01) when measured in baseline biopsy samples coming from OA patients.

In baseline biopsies a variable percentage (from 10% up to 50%) of chondrocyte profiles visible on sections corresponded to degenerating or necrotic cells. As a consequence, the OA tissue exhibited, when compared to the normal control samples, a decrease of more than 40% (P<0.01) in the number of living chondrocytes in both zone I and zone II.

No significant differences were observed in the mean cell volume between samples coming from OA and non-OA patients. The ultrastructure of the chondrocytes, however, underwent significant changes in OA compared to controls

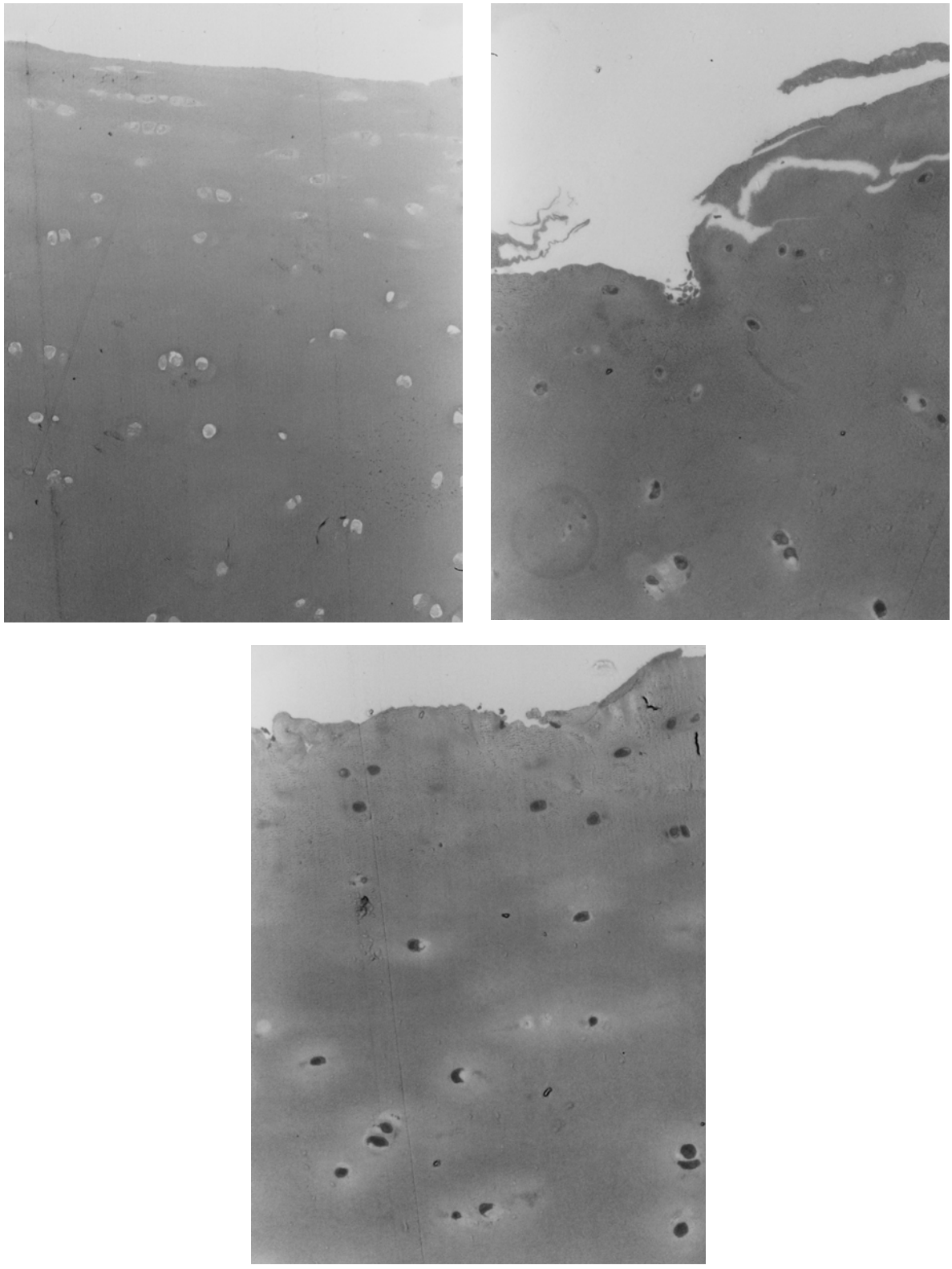

Fig. 2. Photomicrographs (primary magnification: ×20) illustrating the light microscopy appearance of the cartilage samples coming respectively from a non-OA patient (A) and from an osteoarthritic subject at baseline (B) and 6 months after hyaluronan treatment (C).

(Fig. 3). As reported in Table II, a decrease of the cell volume occupied by the organelles responsible for the anabolic metabolism and a correspondent increase of the volume of the structures having catabolic or storage functions were observed in the chondrocytes belonging to OA subjects.

Table II
Morphometric estimators in the articular cartilage of the human knee in primary osteoarthritis at baseline

Parameter	HA	MP	Control	HA vs MP (ANOVA)	OA vs control (ANOVA)
Articular surface:					
RMS	3.13±0.37	2.41±0.42	0.62±0.06	*P*=0.0943	*P*=0.0001
Superficial layer:					
Compactness (score)	0.70±0.18	0.60±0.26	2.26±0.19	*P*=0.7670	*P*=0.0001
Thickness (μm)	0.22±0.07	0.21±0.07	0.59±0.04	*P*=0.8589	*P*=0.0001
Zone I chondrocytes:					
Numerical density (×10^4 cells/mm^3)	0.91±0.14	1.07±0.17	1.84±0.09	*P*=0.4218	*P*=0.0001
Cell volume (μm^3)	783±41	735±29	787±38	*P*=0.4355	*P*=0.5330
RER (μm^3)	29.7±5	35.1±6	69.8±5	*P*=0.5218	*P*=0.0001
Golgi (μm^3)	12.1±2	10.8±3	30.2±4	*P*=0.8277	*P*=0.0002
Mitochondria (μm^3)	8.5±1	7.8±0.7	16.7±1	*P*=0.7011	*P*=0.0001
Lysosomes (μm^3)	3.8±0.8	3.6±0.6	1.24±0.2	*P*=0.8400	*P*=0.0001
Glycogen (μm^3)	18.6±4	21.0±4	8.1±2	*P*=0.6286	*P*=0.0032
Lipid vacuoles (μm^3)	10.7±3	17.9±5	3.8±1	*P*=0.1380	*P*=0.0054
Filaments (μm^3)	31.8±8	29.7±5	7.6±1	*P*=0.8230	*P*=0.0001
Zone II chondrocytes:					
Numerical density (×10^4 cells/mm^3)	0.53±0.04	0.48±0.04	0.86±0.07	*P*=0.6004	*P*=0.0001
Cell volume (μm^3)	802±35	801±34	867±58	*P*=0.9903	*P*=0.2811
RER (μm^3)	29.8±4	42.1±4	78.5±6	*P*=0.1466	*P*=0.0001
Golgi (μm^3)	12.3±1	12.0±2	49.4±8	*P*=0.9768	*P*=0.0001
Mitochondria (μm^3)	7.8±1	8.4±0.9	16.8±0.9	*P*=0.6896	*P*=0.0001
Lysosomes (μm^3)	4.5±0.3	4.3±0.9	1.9±0.3	*P*=0.8349	*P*=0.0002
Glycogen (μm^3)	32.9±6	30.4±9	21.4±5	*P*=0.8138	*P*=0.2041
Lipid vacuoles (μm^3)	12.1±3	15.9±4	7.5±3	*P*=0.5357	*P*=0.0730
Filaments (μm^3)	34.8±2	43.2±5	19.1±3	*P*=0.1416	*P*=0.0001

Mean value±standard error within each group of subjects and significance level.

AFTER TREATMENT FINDINGS

At the final control (on day 180) a reduction of the typical osteoarthritic lesions was observed in about 80% of the second biopsies. Some fissure and fibrillations of the articular surface were still present, but the RMS parameter had improved, exhibiting changes to the value estimated in normal control samples (Table III). When compared with the baseline conditions, however, this change failed to reach the statistical significance in the MP treated group (*P*=0.275) while a statistically significant difference was observed in the HA treated subjects (*P*=0.064).

The superficial amorphous layer (Table III) appeared improved in its structure with both treatments. The compactness score exhibited more pronounced changes towards normality after HA treatment (*P*=0.005) compared to MP (*P*=0.758), but the difference between groups was not statistically significant. Furthermore, only in the HA treated patients was a significant recovery of the thickness of the amorphous layer observed with respect to the basal situation (*P*=0.002) and the difference between the two treatments was also statistically significant (*P*=0.019).

The numerical density of metabolically active chondrocytes (Fig. 4), which was strongly reduced at the basal inspection, appeared partially restored after both treatments. In zone I this recovery reached statistical significance only in the HA treated patients (*P*=0.004), while in zone II the increase exhibited by the parameter when compared with the baseline was significant with both treatments, HA being more effective than MP (*P*=0.002 and *P*=0.049 respectively).

No changes were detected after either treatment in the mean volume of the chondrocytes either in zone I or in zone II. However, a significant increase of the cell volume occupied by structures belonging to the anabolic system and a correspondent significant decrease of the one occupied by organelles with catabolic or storage functions occurred after HA treatment in both zone I and zone II, approaching the reference values observed in the control group. For all these parameters HA proved more effective than MP. As shown in Fig. 5, for most of the ultrastructural parameters significant differences between the two treatments were also observed.

The frequency of occurrence of atypical collagen fibers decreased after treatment. In about 50% of the second biopsies this parameter appeared improved, with no significant difference between the two groups.

A significant difference between treatments (*P*=0.02) was detected when the territorial matrix was considered (Table IV). This parameter showed an improvement in 80% of the biopsies coming from HA treated patients, while only 30% of the MP treated subjects improved and about 50% of them showed a worsening of the territorial matrix appearance.

Discussion

The ability to perform repeated histological examinations of the human cartilage tissue at the same site allowed a detailed analysis of the tissue over time. Furthermore, it may be pointed out that all the observations were performed under blind conditions using, wherever possible, a quantitative approach based on the estimation of the most widely accepted parameters in the literature.

In agreement with already published data[24,30], the data presented here showed that in primary OA of the human knee the articular cartilage was characterized by important

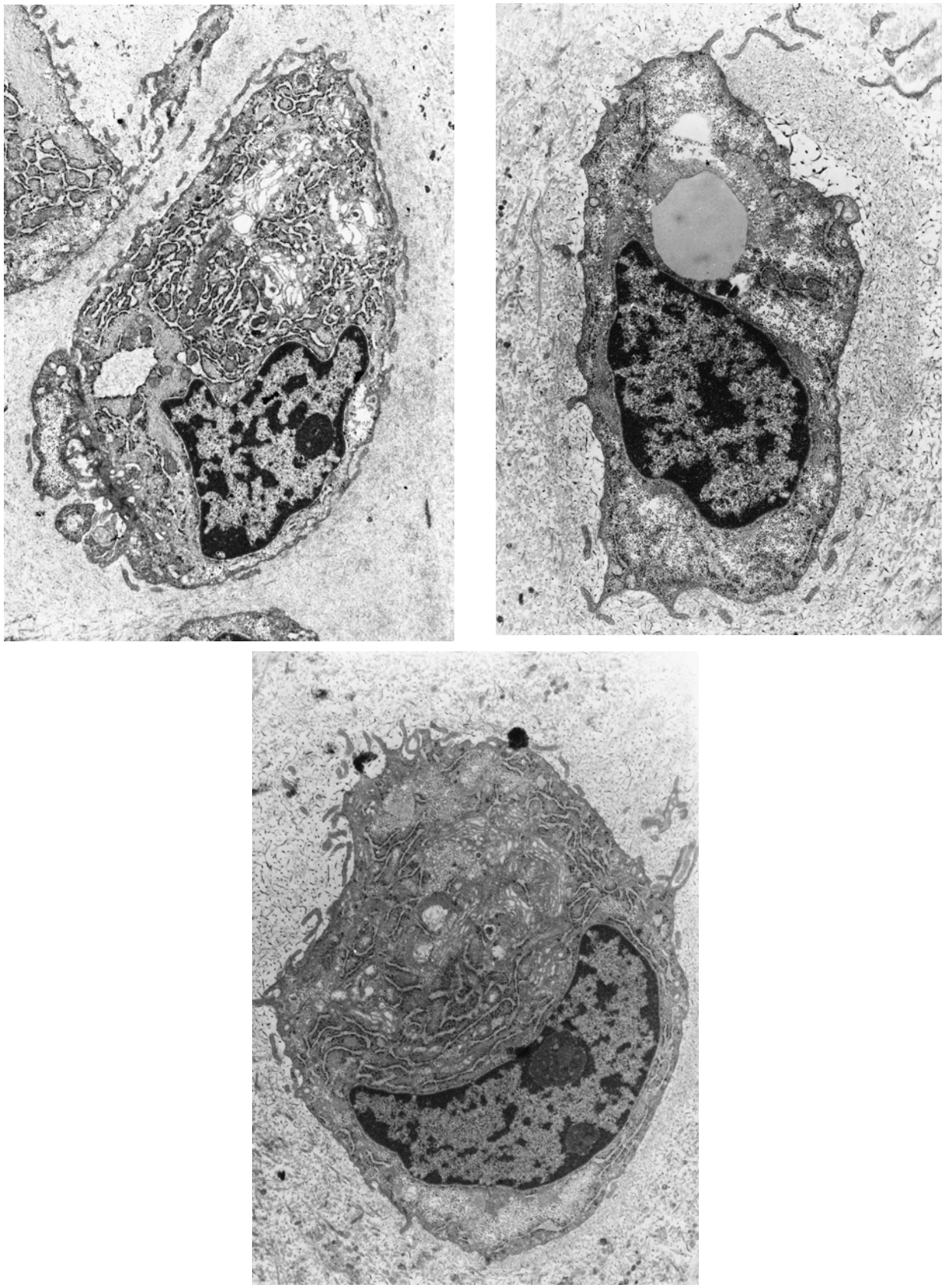

Fig. 3. Ultrastructure of a chondrocyte from a normal subject (A) showing highly-developed RER and Golgi. In (B) is illustrated the ultrastructural appearance of a chondrocyte from an osteoarthritic patient at baseline: the cytoplasm displays abundance of storage structures (lipid vacuoles) and a few cell organelles. Six months after Hyaluronan treatment (C) chondrocytes from the same patient exhibit changes toward normality in their ultrastructure.

Table III
Change from baseline of the morphometric estimators describing the articular surface of the cartilage

Parameter	Treatment	Value	Final vs basal (Sgn Rank test)	HA vs MP (ANOVA)
RMS	HA	−1.11±0.5	P=0.0645	P=0.5326
	MP	−0.70±0.5	P=0.2754	
Superficial amorphous layer:				
Compactness (score)	HA	0.70±0.22	P=0.0050	P=0.2710
	MP	0.25±0.33	P=0.7580	
Thickness (μm)	HA	0.28±0.06	P=0.0020	P=0.0190
	MP	0.02±0.08	P=0.7340	

Mean change from baseline±standard error within each group of subjects and significance level.

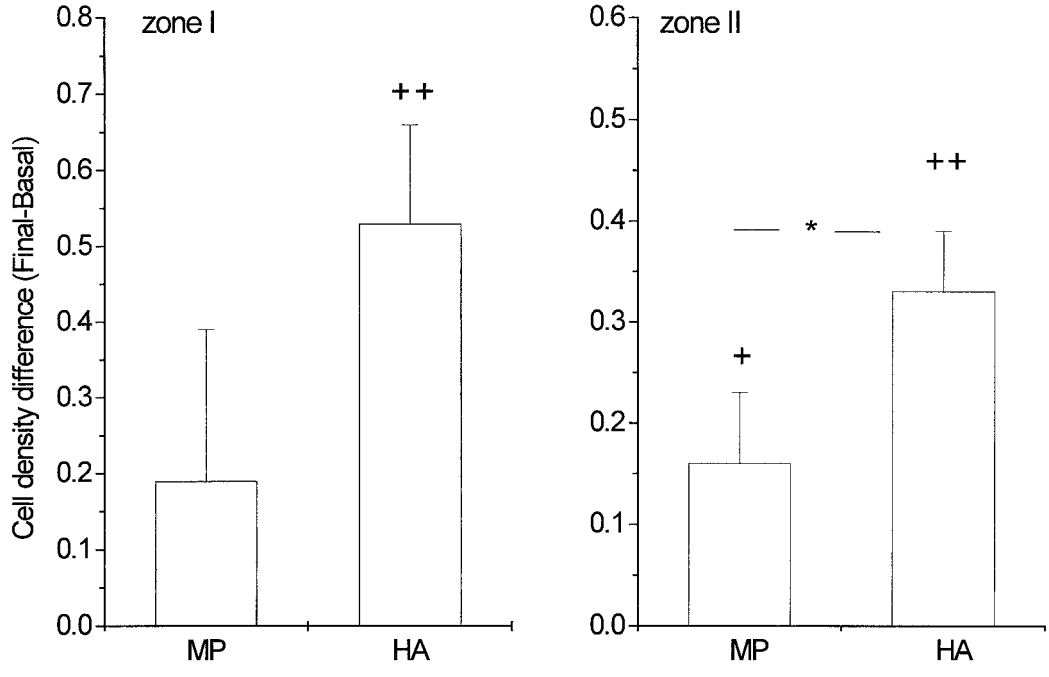

Fig. 4. Numerical density of chondrocytes in zone I and zone II of the articular cartilage after treatment. Values are changes from baseline±standard error. +=P<0.05, ++=P<0.01 (final vs baseline); *=P<0.10 (HA vs MP).

structural modifications. Evidence of articular cartilage degeneration includes the appearance of abnormal histomorphometric features such as increased surface roughness with cleft formation and substantial disaggregation of the amorphous protective layer covering the articular surface. Furthermore, as previously reported in both humans[22] and in animal models of OA[19], the baseline cartilage biopsies from osteoarthritic patients exhibited a significant reduction of the cell density, more pronounced in the region proximal to the articular surface, and a sort of metabolic switching of the surviving chondrocytes towards a more catabolic activity, as indicated by the changes in the ultrastructure of the cells[27].

Adult articular cartilage has only a very limited capacity of spontaneous repair. Such a capacity is limited to full-thickness defects, while partial-thickness defects, analogous to the cleft and fissures seen during early stages of human OA, do not heal spontaneously[31].

Under appropriate conditions, however, a reduction of the lesions was induced. Growth factors, such as IGF-I and TGF-β, proved to be useful[32] in effecting some repair of experimentally created partial-thickness defects. Radiological indications of some cartilage repair were found in patients with severe ankle OA who underwent joint distraction[33], a treatment characterized by the absence of mechanical stress and associated with an improvement of chondrocyte metabolism[34].

Intraarticular corticosteroids remain widely used for symptomatic treatment of peripheral joint osteoarthritis. Several studies in knee OA have indicated a significant benefit compared with placebo, although they appear to have no long-term effect[35,36].

The quite positive results obtained in the MP treated group of patients in some of the structural parameters we measured are consistent with previously published data, obtained in humans[37] and in experimental models of OA[38–40]. They indicated a protective effect of intra-articular corticosteroids on cartilage lesions, but no significant changes in the ultrastructural features of the chondrocytes. It has been suggested[41] that the capability of the corticosteroids to inhibit the stimulation of protease synthesis induced by inflammatory cytokines could be the basis of their beneficial effect when administered intra-articularly.

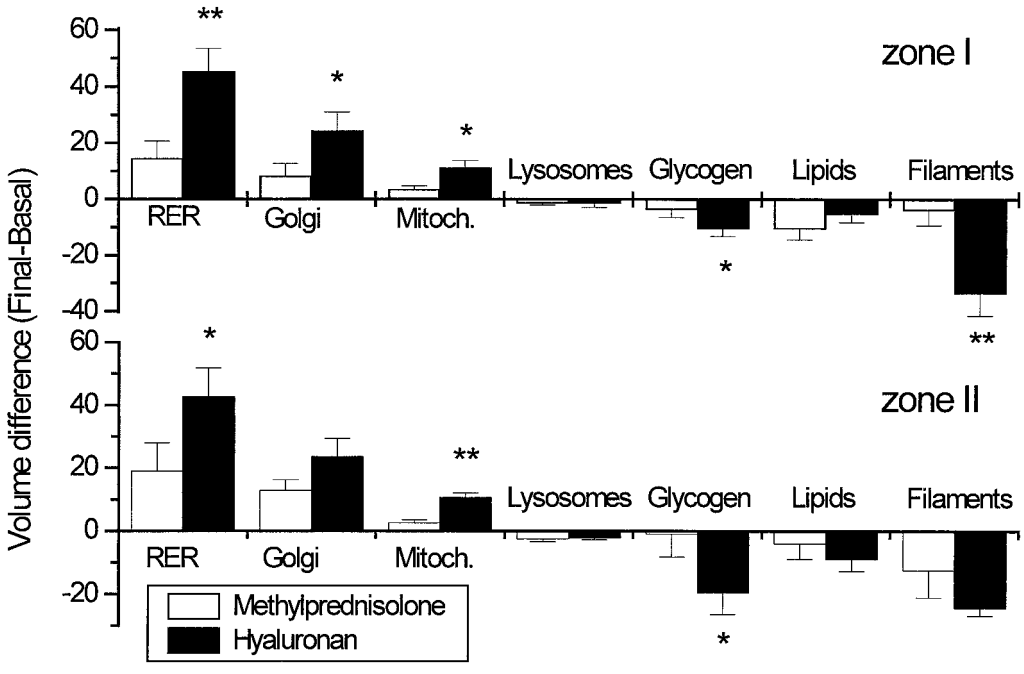

Fig. 5. Morphometric characterization of the ultrastructure of chondrocytes in zone I and zone II of the articular cartilage. Values are changes from baseline±standard error. *=P<0.10, **=P<0.05 (HA vs MP).

Table IV
Changes in terms of modifications in the territorial matrix appearance at month 6 compared with baseline

	HA	MP
Worsened or unchanged	2	9
Improved	9	4

Values are number of patients showing change.

HA treatment, however, proved superior to steroid treatment in almost all the morphometric estimators evaluated in this study. The obtained results are consistent with previously reported clinical data showing a reduction of several markers of cartilage breakdown[42] and a decrease in extension and/or arthroscopic grading of the cartilage lesions[21,22] following intraarticular treatment with 500–730 kDa hyaluronan.

An interesting finding of the present study was the significant improvement of the cellular features. Six months after treatment the number of viable chondrocytes was significantly increased, approaching the value observed in the normal control tissue even in the superficial and more damaged region, whereas no changes of this parameter were observed in the MP treated patients. More interestingly, the cells appeared significantly improved in their metabolism, showing a shift towards a more anabolic activity, as indicated by the increased extension of the synthetic cytocavitary network and mitochondria with respect to the structures having catabolic or storage functions. The observed reduction after HA treatment in the presence of atypical pericellular matrix further supports this improved metabolic activity of the chondrocytes.

Another finding of the present study was the significant improvement, both in structure and thickness, of the superficial amorphous layer of the cartilage 6 months after HA treatment. Since this structure is mainly composed of endogenous hyaluronic acid synthesized by the synoviocytes this result is consistent with previously reported data indicating that HA treatment can lead to control of the synovial membrane inflammation in terms of infiltration of inflammatory cells and hyperplasia of the synovial lining[22] and can also stimulate the neo-synthesis of hyaluronic acid by the synoviocytes[43].

Altogether the results obtained give further support to the suggestion that intraarticular treatment with 500–730 kDa HA may represent not only an efficacious symptomatic treatment for osteoarthritis of the knee but also a candidate for structure modification.

Acknowledgments

The authors would like to acknowledge statistical support of Dr S. Piva. Helpful suggestions from Dr L. Beinat, Dr C. Bevilacqua and Dr M. O'Regan are also gratefully acknowledged.

References

1. Dieppe P. Osteoarthritis: risk factors, process and outcome. Rheumatology Europe 1995;24:66–8.
2. Lequesne M, Brandt K, Bellamy N, Moskowitz R, Menkes CJ, Pelletier JP. Guidelines for testing slow-acting drugs in osteoarthritis. J Rheumatol 1994;21(Suppl):65–71.
3. GREES (Group for the Respect of Ethics and Excellence in Science—Osteoarthritis section). Recommendations for the registration of drugs used in the treatment of osteoarthritis. Ann Rheum Dis 1996;55:552–7.
4. Guerra D, Frizziero L, Losi M, Bacchelli B, Mezzadri G, Pasquali-Ronchetti I. Ultrastructural identification of a membrane-like structure on the surface of normal

articular cartilage. J Submicrosc Cytol Pathol 1996;28:385–93.
5. Swann DA, Redin TL, Navimien M, Weisser PA, Curran N, Lewinnek G. Role of hyaluronic acid in joint lubrication. Ann Rheum Dis 1974;33:318–26.
6. Balazs EA. Physical properties of synovial fluid. In: Helfet A, Ed. Disorders of the Knee. Philadelphia: Lippincot Co 1974:63–73.
7. Dahl LB, Dahl IM, Enstrom-Laurent A, Granath K. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Ann Rheum Dis 1985;44:817–22.
8. Rydell NW, Butler J, Balazs EA. Hyaluronic acid in synovial fluid. VI. Effect of intra-articular injection of hyaluronic acid on the clinical symptoms of arthritis in track horses. Acta Vet Scand 1970;11:139–55.
9. Peyron JG, Balasz EA. Preliminary clinical assessment of NA-hyaluronate injection into human arthritic joint. Pathol Biol 1974;22:731–6.
10. Weiss C, Balazs EA, St Onge R, Denlinger JL. Clinical studies of the intra-articular injection of Healon® (sodium hyaluronate) in the treatment of osteoarthritis of human knee. Semin Arthritis Rheum 1981;11(Suppl 1):143–4.
11. Peyron JG. A new approach to the treatment of osteoarthritis: viscosupplementation. Osteoarthr Cartil 1993;1:85–7.
12. Aviad AD, Houpt JB. The molecular weight of therapeutic hyaluronan (sodium hyaluronate) how significant is it? J Rheumatol 1994;21:297–301.
13. Carabba M, Paresce E, Angelini M, Re KA, Torchiana EEM, Perbellini A. The safety and efficacy of different dose schedules of hyaluronic acid in the treatment of painful osteoarthritis of the knee with joint effusion. Eur J Rheumatol Inflamm 1995;15:25–31.
14. Scali JJ. Intra-articular hyaluronic acid in the treatment of osteoarthritis of the knee: a long term study. Eur J Rheumatol Inflamm 1995;15:57–62.
15. Rydell NW, Balazs EA. Effect of intra-articular injection of hyaluronic acid on the clinical symptoms of osteoarthritis and granulation tissue formation. Clin Orthop 1971;80:25–32.
16. Kolarz G, Kotz R, Broll H, Dunky A, Landsiedl F, Mayrhofer F, *et al*. Hyaluronic acid in the treatment of osteoarthritis of the knee joint: interim results of a comparative clinical study. Eur J Rheumatol Inflamm 1995;15:39–45.
17. Altman RD, Moskowitz R. Intraarticular Sodium Hyaluronate (Hyalgan®) in the treatment of patients with osteoarthritis of the knee: a randomized clinical trial. J Rheumatol 1998;25:2203–12.
18. Abatangelo G, Botti P, Del Bue M, Gei G, Samson J, Cortivo R, *et al*. Intra-articular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs. I. Biochemical results. Clin Orthop Rel Res 1989;241:278–85.
19. Schiavinato A, Lini E, Guidolin D, Pezzoli G, Botti P, Martelli M, *et al*. Intra-articular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs. II. Morphological findings. Clin Orthop Rel Res 1989;241:286–99.
20. Ghosh P, Burkhardt D, Read R, Bellenger C. Recent advances in animal models for evaluating chondroprotective drugs. J Rheumatol 1991;18:143–146.
21. Listrat V, Xavier A, Patarnello F. Arthroscopic evaluation of potential structure modifying activity of hyaluronan (Hyalgan) in osteoarthritis of the knee. Osteoarthritis Cartil 1997;5:153–60.
22. Frizziero L, Govoni E, Bacchini P. Intra-articular hyaluronic acid in the treatment of osteoarthritis of the knee: Clinical and morphological study. Clin Exp Rheumatol 1998;16:441–9.
23. Altman RD, Asche E, Bloch D. Development of criteria for the classification and reporting of osteoarthritis. Arthritis Rheum 1986;29:1039–49.
24. Ghadially FN. Fine Structure of the Synovial Joints. London: Butterworths 1983.
25. Eggli PS, Hunziker EE, Schenk RK. Quantitation of structural features characterizing weight- and less weight-bearing regions in articular cartilage: a stereological analysis of medial femoral condyles in young adult rabbits. Anat Rec 1988;222:217–27.
26. Hacker SA, Healey RM, Yoshioka M, Coutts RD. A methodology for the quantitative assessment of articular cartilage histomorphometry. Osteoarthritis Cart 1997;5:343–55.
27. Annefeld M. A new test method for the standardized evaluation of changes in the ultrastructure of chondrocytes. Int J Tiss Reac 1985;7:273–89.
28. Weibel ER. Stereological Methods. Vol. I. London: Academic Press 1979.
29. Winer BJ. Statistical Principles in Experimental Design. Tokyo: McGraw-Hill 1971.
30. Carrabba M, Colombo B, Govoni E, Cenacchi G. Ultrastructural approach to the understanding of osteoarthrosis. Rheumatology 1982;7:53–63.
31. Hunziker EB. Articular cartilage repair: are the intrinsic biological constraints undermining this process insuperable? Osteoarthritis Cartil 1999;7:15–28.
32. Hunziker EB, Rosenberg LC. Induction of repair in partial-thicness articular cartilage lesions by timed release of TGF-β (Abstract). Trans Orthop Res Soc 1994;236–41.
33. Van Valburg AA, van Roermund PM, Marijnissen ACA, van Melkebeek J, Lammens J, Verbout AJ, *et al*. Joint distraction in treatment of osteoarthritis: a two-year follow-up of the ankle. Osteoarthritis Cartil 1999;7:474–79.
34. Van Valburg AA, van Roermund PM, Marijnissen ACA, Wenting JG, Verbout AJ, Lafeber FPJG, *et al*. Joint distraction in treatment of osteoarthritis (II): effects on cartilage in a canine model. Osteoarthritis Cart 2000;8:1–8.
35. Creamer P. Intra-articular corticosteroid treatment in osteoarthritis. Curr Opin Rheumatol 1999;11:417–21.
36. Ravaud P, Moulinier L, Giraudeau B, Ayral X, Guerin C, Noel E, *et al*. Effects of joint lavage and steroid injection in patients with osteoarthritis of the knee: results of a multicenter, randomized, controlled trial. Arthritis Rheum 1999;42:475–82.
37. Pelletier JP, Martel-Pelletier J, Malemud CJ, Woessner JF Jr. Proteoglycan-degrading acid metalloprotease activity in human osteoarthritic cartilage and the effect of intraarticular steroid injections. Arthritis Rheum 1987;30:541–8.
38. Pelletier JP, Martel-Pelletier J. Protective effects of corticosteroids on cartilage lesions and osteophyte formation in the Pond-Nuki dog model of osteoarthritis. Arthritis Rheum 1989;32:181–93.
39. Pelletier JP, DiBattista JA, Raynauld JP, Wilhelm S, Martel-Pelletier J. The in vivo effects of intraarticular corticosteroid injections on cartilage lesions, stromelysin, interleukin-1, and oncogene protein synthesis

in experimental osteoarthritis. Lab Invest 1995;72: 578–86.
40. Williams JM, Brandt KD. Triamcinolone hexacetonide protects against fibrillation and osteophyte formation following chemically induced articular cartilage damage. Arthritis Rheum 1985;28:1267–74.
41. Caron JP, Tardif G, Martel-Pelletier J, DiBattista JA, Geng C, Pelletier JP. Modulation of matrix metalloprotease 13 (collagenase 3) gene expression in equine chondrocytes by interleukin 1 and corticosteroids. Am J Vet Res 1996;57:1631–34.
42. Creamer P, Sharif M, George E, Meadows K, Cushnaghan J, Shinmei M, *et al*. Intra-articular hyaluronic acid in osteoarthritis of the knee: an investigation into mechanisms of action. Osteoarthr Cart 1994;2:133–40.
43. Smith MM, Ghosh P. The synthesis of hyaluronic acid by human synovial fibroblasts is influenced by the nature of the hyaluronate in the extracellular environment. Rheumatol Int 1987;7:113–22.