OsteoArthritis and Cartilage (2003) **11,** 845–853
© 2003 OsteoArthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/S1063-4584(03)00162-6

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Characterisation of the guinea pig model of osteoarthritis by *in vivo* three-dimensional magnetic resonance imaging

J. J. Tessier Ph.D.†*, J. Bowyer B.Sc.‡, N. J. Brownrigg B.Sc.‡, I. S. Peers Ph.D.†, F. R. Westwood Ph.D., FRCPath§, J. C. Waterton Ph.D.† and R. A. Maciewicz Ph.D.‡

† *Enabling Science and Technology, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

‡ *Respiratory and Inflammation, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

§ *Safety Assessment, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

Summary

Objective: To characterise longitudinal changes in joint integrity and cartilage volume *in vivo* in the guinea pig spontaneous osteoarthritis (OA) model by magnetic resonance imaging (MRI).

Methods: Guinea pigs knee were imaged *in vivo* by high-resolution three-dimensional (3D) MRI between the ages of 3 and 12 months. Image analysis was performed to assess qualitative knee joint changes between 3 and 12 months ($n=16$) and quantitative volumetric changes of the medial tibial cartilage between 9 and 12 months ($n=7$). After imaging, animals were killed and knees were assessed macroscopically and histologically.

Results: From 3 to 6 months qualitative observation by MRI and histopathology indicated localised cartilage swelling on the medial tibial plateau. At 6 months, bone cysts had developed in the epiphysis. At 9 months, we observed by MRI and histopathology, fragmentation of the medial tibial cartilage in areas not protected by the meniscus. Cartilage degeneration had intensified at 12 months with evidence of widespread loss of cartilage throughout the tibial plateau. Segmentation of the MR cartilage images showed a 36% loss of volume between 9 and 12 months.

Conclusions: We have achieved 3D image acquisition and segmentation of knee cartilage in a guinea pig model of chronic OA, which permits measurements previously only possible in man. High resolution and short acquisition time allowed qualitative longitudinal characterisation of the entire knee joint and enabled us to quantify for the first time longitudinal tibial cartilage volume loss associated with disease progression.
© 2003 OsteoArthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Key words: Osteoarthritis, Guinea pig, MRI, Cartilage volume.

Introduction

Osteoarthritis (OA) is one of the most common and disabling disorders in the general population. It is a slowly progressive disease, characterised morphologically by destruction of cartilage, formation of bone cysts, sclerosis of subchondral bone and presence of osteophytes at the joint margin. There is a large unmet medical need for therapeutics to treat OA. The development of any new therapy is facilitated by proof of concept and proof of principle studies in animal models[1–3].

Because aetiology of OA is not fully understood, it is difficult to evaluate how well any animal model of OA mimics the disease in humans[3]. However, Dunkin Hartley guinea pigs develop spontaneous OA of the knee and the resulting articular cartilage lesions resemble those found in humans[4]. Similar to humans, the guinea pigs develop age-related OA and the incidence of the disease is increased by body weight, mechanical load and posture[4–6]. It has been shown histologically that the disease in Dunkin Hartley preferentially affects the medial side possibly due to obesity and the *varus* position the animal adopts[7–9]. However, since the disease develops spontaneously and asymptomatically in this strain, it is difficult to determine precisely the onset, the extent of the disease, and inter-animal variation. With such variability, it is therefore more desirable to monitor intra-animal disease progression.

Magnetic resonance imaging (MRI) is a powerful technique in which to do this, as it is sensitive and suited to detect compositional change and integrity of the whole knee joint including articular cartilage, bone, meniscus, tendons and ligaments. Furthermore, MRI trials assessing the efficacy of disease-modifying osteoarthritis drugs (DMOADs) in causing changes in human knee joints may have greater statistical power than equivalent trials using conventional X-ray joint-space narrowing. Of five recent MRI studies[10–14] of cartilage volume progression in OA, four[11–14] detected loss with up to 2 years observation, and one study[11] of 35 subjects detected loss with only 6 months observation. In contrast, it has been estimated that one would have to study several hundred patients for 2–3 years to have any chance of detecting drug effects on structural changes in OA using X-radiography[15]. However, validation of MRI endpoints for OA trials is still incomplete, and, unlike X-radiography[16–18], no MRI study has previously demonstrated the effect of a disease-modifying anti-OA agent in

*Address correspondence and reprint requests to: Jean J. Tessier. Tel: 44-1625-515257; Fax: 44-1625-514463; E-mail: jean.tessier@astrazeneca.com

Received 13 January 2003; revision accepted 7 July 2003.

slowing cartilage loss. The design and interpretation of a clinical trial is immeasurably strengthened if the effect of the drug is shown on the same endpoint in a valid animal model.

MR imaging has already been applied to a variety of OA animal models, including mouse[19], rat[20], guinea pig[21], rabbit[22], monkey[23], goat[24] and dog[25]. Disease progression of the guinea pig model of OA previously has been characterised by histology[3,26,27] and by two-dimensional (2D) MRI[21], which showed focal changes in the articular cartilage of the medial tibial plateau. However, a full appreciation of the extent of the disorder requires a complete assessment of the knee joint. To our knowledge, no 3D MRI has been performed *in vivo* on knee joints of animals of this size. Because of the difficulties in obtaining good quality, high-resolution images, acquisition of 3D data set of joints as small as a guinea pig knee has not been considered possible by some authors[21]. For this reason, several research groups have resorted to working in large animals in order to get analysable images[22–25].

In this study, we have undertaken high-resolution 3D MRI on the guinea pig hind knee at several time-points to assess progressive changes within the same knee joint. The images cover the entire knee joint with a high signal-to-noise ratio and are used to quantify for the first time, changes *in vivo* in the articular cartilage volume during progression of the disease.

Methods

ANIMALS

Male Dunkin Hartley guinea pigs (Harlan, UK) between the ages of 3 and 12 months were housed either singly or in pairs with age-matched mates, and allowed food and water *ad libitum*. Prior to imaging, anaesthesia was induced using inhalation anaesthesia (3% (v/v) halothane 'Fluothane' (AstraZeneca, Macclesfield, UK) in 95% O_2/5% CO_2).

Animals were then placed on a Perspex platform with the left hind leg extending through a radio-frequency coil. To prevent motion, the footpad was secured to a secondary lower platform by means of a soft Velcro strap. Guinea pigs were kept anaesthetised for the duration of imaging with 1.5% Fluothane. Gaseous anaesthesia was supplied in the magnet via a facemask.

Respiration rate was monitored continuously during imaging using a signal transducer/amplifier linked to a water-filled balloon placed under the abdomen of the animal. Temperature of the animals was monitored continuously by rectal probe and maintained at 38°C±0.1 by a continuous flow of heated air.

Post-imaging, guinea pigs were placed on a heat mat kept at 37°C until recovery was complete. Once fully recovered they were returned to their animal housing facility.

All work was performed in full compliance with licenses issued under the UK Animals (Scientific Procedures) Act, 1986.

MR IMAGE ACQUISITION

Sixteen guinea pigs were imaged *in vivo* for less than 2 h on a Varian 4.7 T MRI system using a double balanced matched 3 cm diameter copper sheet solenoid, 1 cm in length constructed in this laboratory (180° pulse length=50 μs at 50 W, coil unloaded).

A 3D data set was acquired for 105 min using spoiled fat-suppressed 3D gradient echo (TR=75 ms, TE=2.7 ms, flip angle=30°). Prior to the acquisition of the 3D data set, the overall positioning of the guinea pig was checked using a fast multi-slice sagittal gradient echo image to ensure that the leg was placed adequately within the radio-frequency coil. Then, a transverse image of the knee was used to select the orientation of the sagittal view of the 3D images such that they were parallel to the medial condyle.

Homogenising the main magnetic field, a process known as 'shimming', was facilitated by the small volume of the coil, giving a half-width water linewidth of around 100 Hz. This allows complete fat suppression in the centre of the coil using an 8.2 ms Gaussian pulse placed 650 Hz off-resonance from the water signal. The image matrix was 512×192×96 points and the spectral width of the MR images was set to 71 685 Hz, corresponding to 140 Hz per point. The length and diameter of the copper sheet solenoid radio-frequency coil used in this study were optimised to minimise the image field of view (30×30×30 mm), while maintaining adequate signal-to-noise. The image completely covered the knee joint.

Implementation of an asymmetric echo allowed a decrease in the imaging echo time to 2.7 ms and an increase in the signal-to-noise of the images.

IMAGE PROCESSING

The data acquired were processed and analysed on a SUN Ultra 60 using software developed in-house and written in IDL (Research Systems Inc., Boulder, CO, USA). The image matrix was first zero-filled to 512×256×128 to obtain after 3D Fourier transform, an apparent image resolution of 59×117×234 μm. The highest resolution (59 μm) was chosen to be across the cartilage thickness (300–350 μm at 9 months). The 3D images were displayed using a multiple view environment as shown in Fig. 1, which permits the simultaneous visualisation along the sagittal, coronal and transversal orientations.

Bone, meniscus and cartilage appearances were evaluated qualitatively. For bone, this was done by locating osteophytes and cysts. For menisci and tibial cartilage, the extent of fragmentation and intensity changes were determined. The intensity variations were scored high, medium or low based on comparison with muscle signal, which was assumed constant. In addition, the medial cartilage swelling was assessed by direct comparison with the lateral side.

Segmentation of the cartilage of the medial plateau was performed on sagittal slices covering the medial side only. These slices were pre-selected by one experienced observer on the basis that they covered the 'flattest' region of the medial tibial surface giving the clearest images. The slices covering the inner side where the cruciate ligament is attached were excluded because the images are subject to significant partial volume averaging. Slices at one time-point were matched as close as possible to those obtained at subsequent time-points based on image references, e.g., cysts and orientation. Thus for any animal, the number of slices analysed were equal for all time-points. However, the number of segmented slices was different between animals (between seven and nine) to account for the difference in the overall size of the tibial plateau. Before segmenting, an experienced user adjusted the image grey scale and zoom to increase the contrast between the

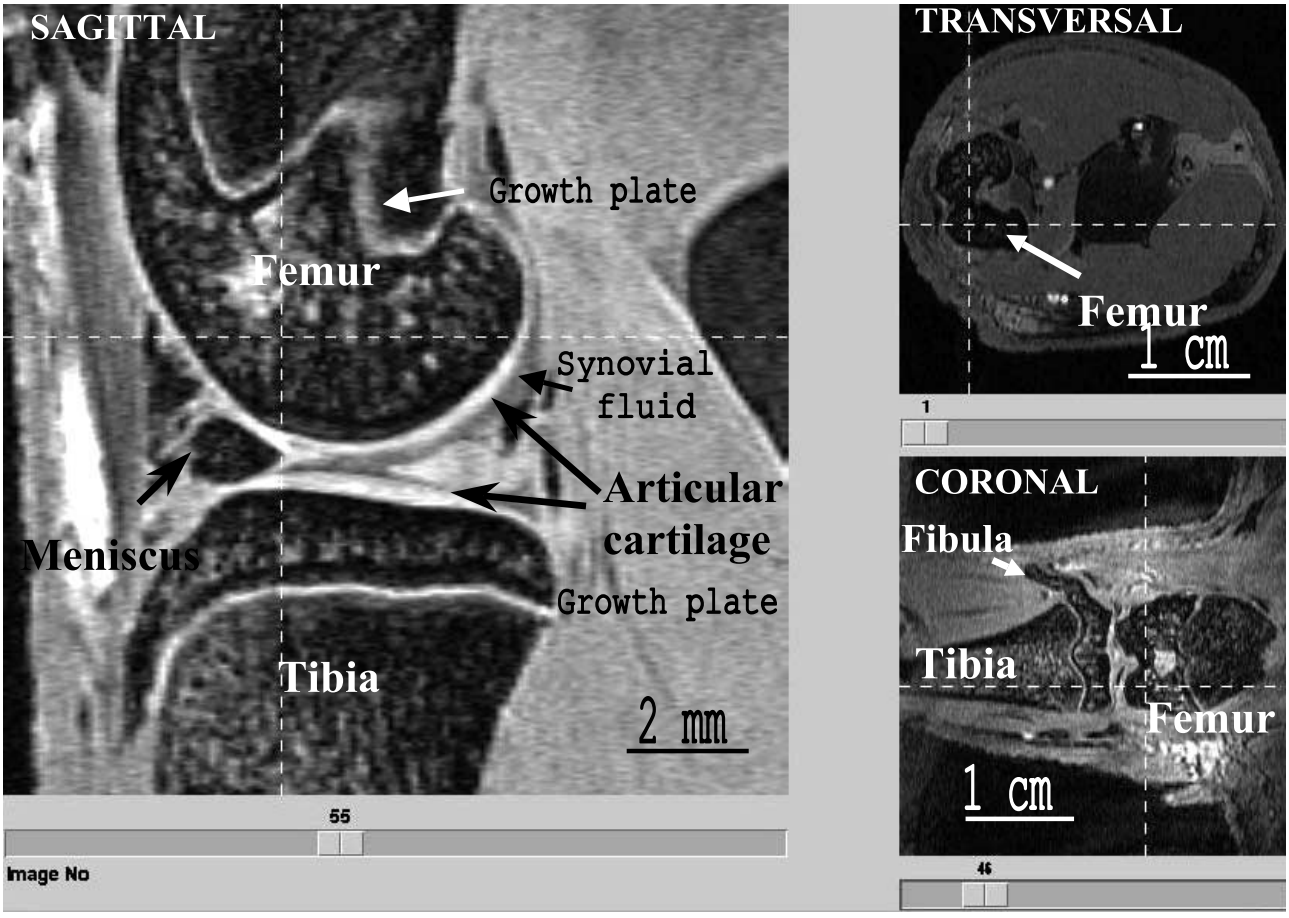

Fig. 1. Interface written on IDL, to visualise simultaneously the sagittal, coronal and transversal views of the 3D guinea pig knee images. The 3D MRI sequence was optimised in order to obtain the maximum contrast between the cartilage (bright) and the surrounding synovial fluid and bones (dark). The guinea pig tibial plateau is less than 1 cm in length, i.e., more than an order of magnitude smaller than in humans. The cartilage volume is three orders of magnitude smaller in the guinea pig.

cartilage and the surrounding tissues. This setting was stored so that subsequent segmenters applied the same image grey scale and zoom.

Segmentation was done manually by selecting points on the cartilage surface. The points were joined automatically using an Akima spline interpolation[28] and the volume of the region selected was calculated. Before segmenting the cartilage, the images were renamed, so that the observers were blinded to the animal identity and age but were aware of the slices to segment for any given animal.

STATISTICAL ANALYSIS

Three observers performed cartilage segmentation on seven guinea pigs at 9, 10.5 and 12 months. No cartilage segmentation was performed at 3 and 6 months, because the animals were not skeletally mature and histology shows only mild cartilage degeneration. Statistical analysis was performed on those segmentations to explore the causes of the variability (i.e., components of variance), and establish the significance of the changes seen (effect sizes and 95% confidence intervals).

HISTOPATHOLOGY

At 3, 6 and 9 months, three animals were killed in accordance with Schedule 1 of the UK Animals (Scientific Procedures) Act 1986 and the left and right knees examined by histopathology. The left knee of the seven remaining guinea pigs was taken for histopathological examination after imaging at 12 months. Whole legs were fixed in 10% (v/v) buffered formalin, and following decalcification in 10% (v/v) formic acid, femorotibial joints with associated tissues were isolated and bisected so that the lateral and medial sides could be processed by standard histological techniques and embedded in wax separately. Approximately five steps sections (5 μm) were prepared through the whole joint sample in each wax block, stained with haematoxylin and eosin, and examined by light microscopy for degenerative and proliferative changes. These included chondrocyte loss and clustering, cartilage fragmentation and erosion, and bone osteophytes.

MACROSCOPIC ASSESSMENT

Macroscopic visualisation of tibial cartilage degeneration by India ink staining was performed as described by Richardson *et al.*[29]. Briefly, the right hind leg (opposite leg to that scanned by MRI) was taken at 12 months from every guinea pig post-study and stored at –20°C. Within 1 month, the guinea pig legs were thawed by immersion in phosphate buffered saline (PBS) pH 7.4 for approximately 20 min. The tibia and femur bones were separated and all excess soft tissue was removed by careful dissection under

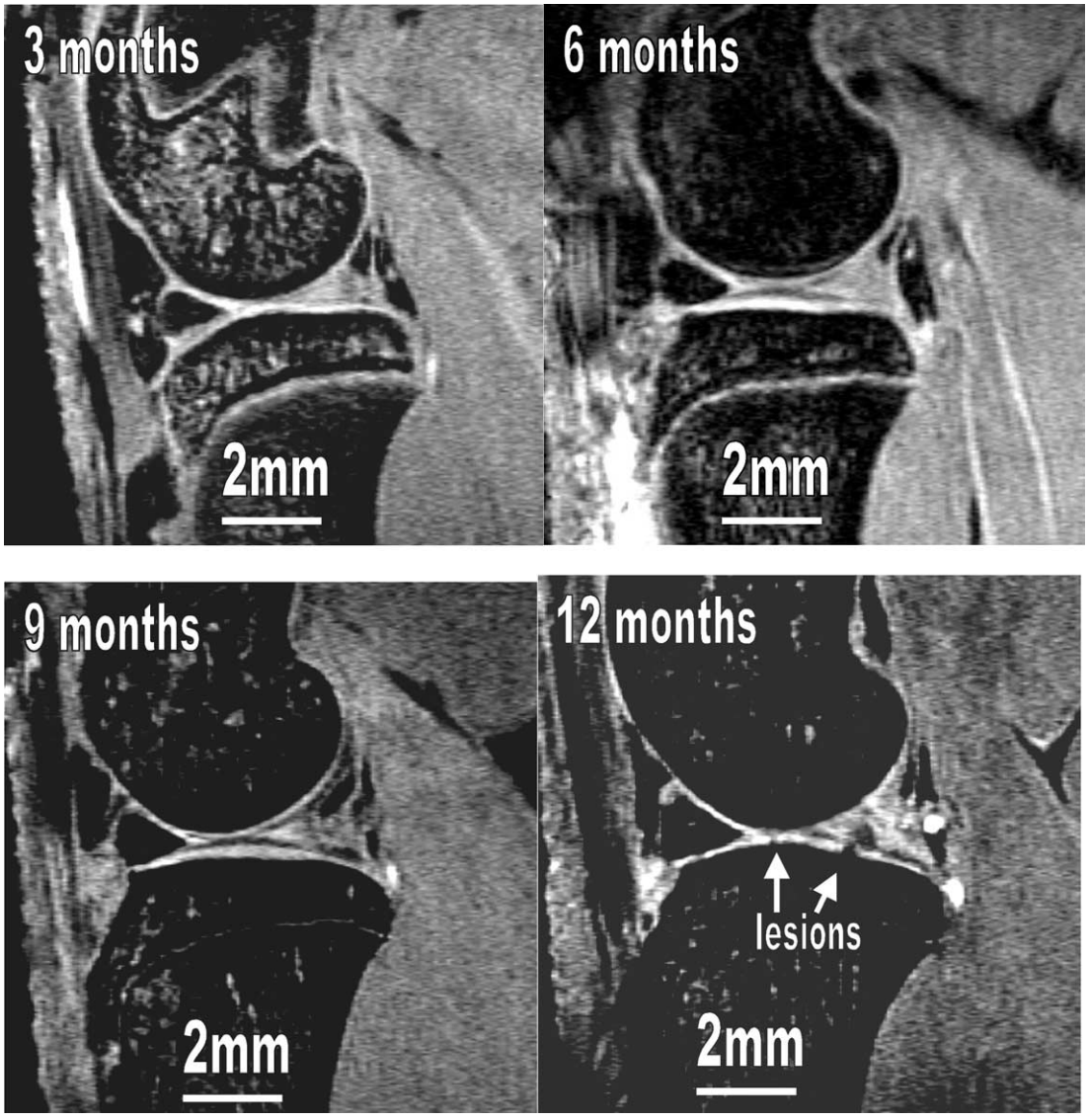

Fig. 2. Sagittal slices through the medial side of 3-, 6-, 9- and 12-month-old guinea pig hind knee joint, showing the changes described in Table I.

a dissecting microscope. Menisci were dissected off in order to expose the tibial cartilage surface. The tibial plateau of each bone then was painted with a 20% (v/v) dilution of blue India ink (Parker, Quink®) blotting any excess off with a moist cotton swab. After 15 s, images of the stained tibial plateau were then captured using an Hitachi HV-C20 camera. Image analysis was done using Paint Shop Pro and Data Cell Snapper Tool software programs. Area of damage was expressed as a percentage of the medial tibial plateau area.

Results

High-resolution, high signal-to-noise *in vivo* 3D MR images of the guinea pig knee were obtained in order to characterise OA progression. Cartilage integrity, bone and meniscus changes between 3 and 12 months could be visualised (Fig. 2) and are summarised in Table I.

At 3 months, the joint appeared macroscopically and histologically normal [Fig. 3(A,C)]. Compared with the lateral side, MRI showed localised articular cartilage swelling on the medial tibial plateau. The bone marrow in the epiphysis was bright with no evidence of cysts.

By 6 months, the medial tibial plateau cartilage was swollen in all animals and bone cysts had developed in the central region of the tibial plateau. Histology confirmed the presence of epiphyseal cysts.

At 9 months, MR images showed the presence of large epiphyseal bone cysts and marked fragmentation of the medial tibial cartilage in the areas not covered by the meniscus together with pronounced cartilage lesions. Histology confirmed the presence of these changes including epiphyseal cysts and articular cartilage thickening and degeneration. A representation of this change is shown in Fig. 3D where erosion of the cartilage exists side by side with cartilage thickening. Degenerative changes were characterised by abnormal distribution of chondrocytes

Table I
Cartilage integrity, bone and meniscus changes from 3 to 12 months

Age (months)	3	6	9	12
Mean weight (g)±S.E.M.	550±16	800±15	1150±37.2	1225±45.0
Number of animals	3	3	10	7
Bone: signal intensity in tibial bone epiphysis	High	Medium	Low	Low
Bone cyst (tibia)	Absent	Several small cysts in central region	Large bone in central region	Large bone in central region
Cartilage signal intensity compared to muscle (medial side)	High	Not uniformly bright	Not uniformly bright	Not uniformly bright
Cartilage morphology (medial side)	Localised swelling	Swollen	Swollen fragmented+holes	Swollen fragmented+holes
Growth plate	Large and bright	Apparent	Remnants	Absent
Anterior meniscus	Dark	Dark	Dark	Dark
Posterior meniscus	Bright	Bright with dark patches	Bright with dark patches	Bright with dark patches

At 3 months, we observe localised cartilage swelling on the medial tibial plateau, the bone marrow in the epiphysis is bright with no evidence of cysts. By 6 months, cartilage is swollen in all animals, with bone cysts developing in the central region of the tibial plateau. At 9 months, we observe large bone cysts and marked fragmentation of the medial tibial cartilage in the areas not covered by the meniscus together with pronounced cartilage lesions. At 1 year, focal thinning of the cartilage is apparent with occasional full cartilage loss (Fig. 2).

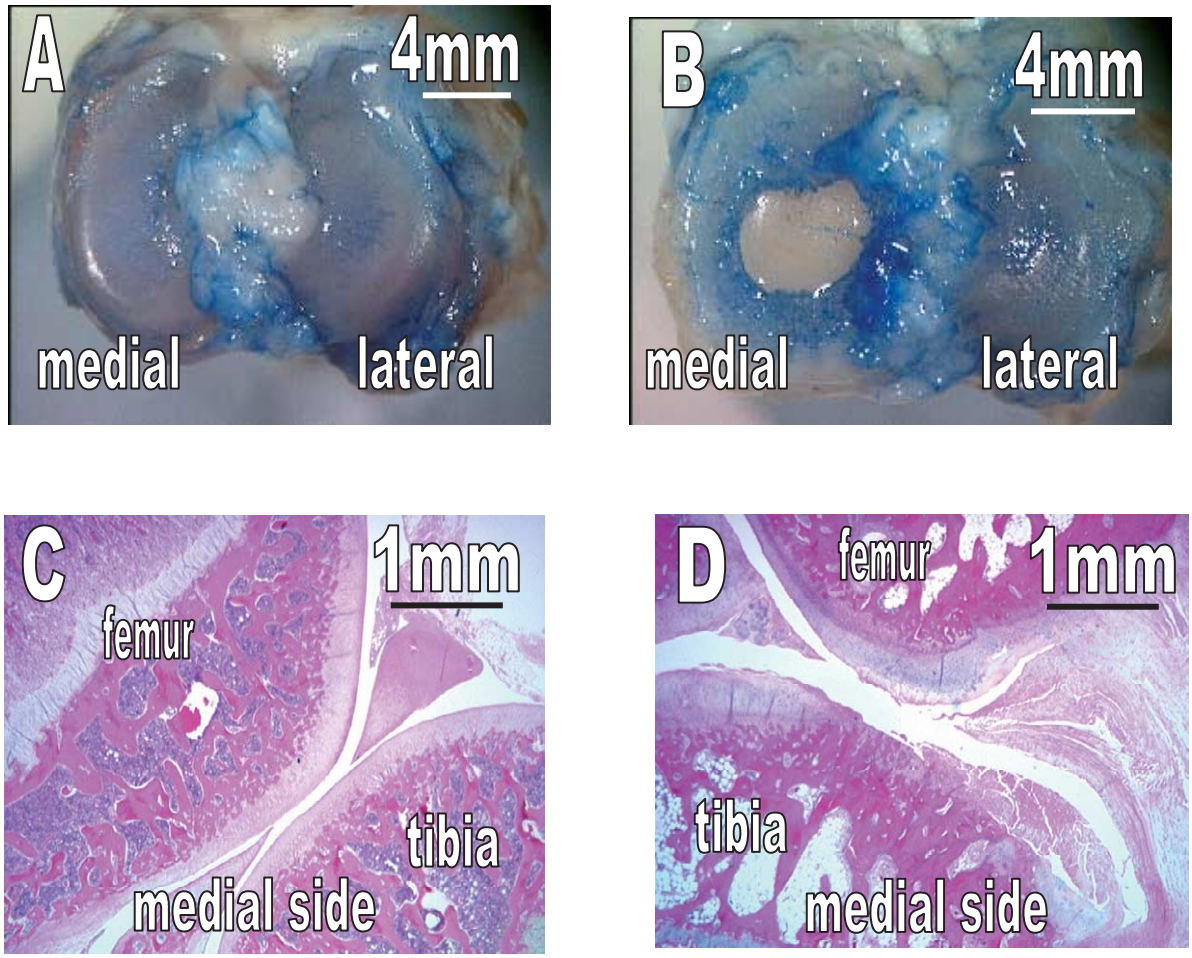

Fig. 3. Macroscopic observation and histology of the tibial cartilage of a 3-month-old (A,C) and 12-month-old (B,D) Dunkin Hartley guinea pig. No cartilage degeneration is observable at 3 months. In contrast, at 12 months, the cartilage is degenerate with erosion and fragmentation on the medial side.

with loss and clustering, cartilage fragmentation and erosion, and cartilage fragments in the joint space. Synovial hyperplasia was also seen. At this age, the growth plate mainly was closed with only remnants visible by imaging (Fig. 2).

At 12 months, macroscopic observation and histology [Fig. 3(B,D)] confirmed the articular cartilage degeneration seen by MRI, particularly in, but not restricted to the medial tibial area. There were extensive degenerative and erosive lesions [Fig. 2(D)] and a corresponding increase in cartilage fragments in the joint space. There were areas of cartilage thickening, thinning and loss with residual matrix showing similar but more severe degenerative changes to those seen at 9 months.

Both MRI and macroscopic anatomical observation showed that the extent of cartilage degeneration varied between animals. Macroscopic assessment revealed areas of pitting and occasionally full thickness loss in the medial tibial cartilage [Fig. 3(B)] with a quarter of the cartilage classified as damaged (average surface pitted=24%, standard deviation=16%, n=7). Most of the cartilage degeneration occurred in the centre of the plateau in the region not protected by the meniscus. Histopathological assessment showed that in addition to these changes the meniscus was also degenerate and that mild synovial hyperplasia with cystic dilatation was present.

At all ages, the anterior meniscus MR images had low signal intensity. The posterior side mainly was bright but dark patches increased with age, indicating a composition difference. Histopathology confirmed this to be meniscal ossification.

The quality of the MR images was sufficient to permit blinded, manual segmentation of the medial tibial plateau cartilage to give a volumetric measurement (Fig. 4). Segmentation was not performed on guinea pigs below 9 months because histology shows only mild cartilage degeneration at 3 and 6 months. In addition, the animals were not skeletally mature at this age and hence the cartilage volume would be affected by the increase in tibial plateau area. This was evident qualitatively not only from the MR images showing open growth plates but also from measuring tibial bone dimensions (data not shown). The mean cartilage volume between 9 and 10.5 months showed a non-significant average decrease of 5.26% (effect size=−0.34, 95% confidence interval −1.39 to +0.72). A statistically significant mean loss of 36% was observed between 9 and 12 months (effect size=−1.47, 95% confidence interval −2.66 to −0.29, P=0.02). Over this 3-month period, all the animals lost cartilage. The

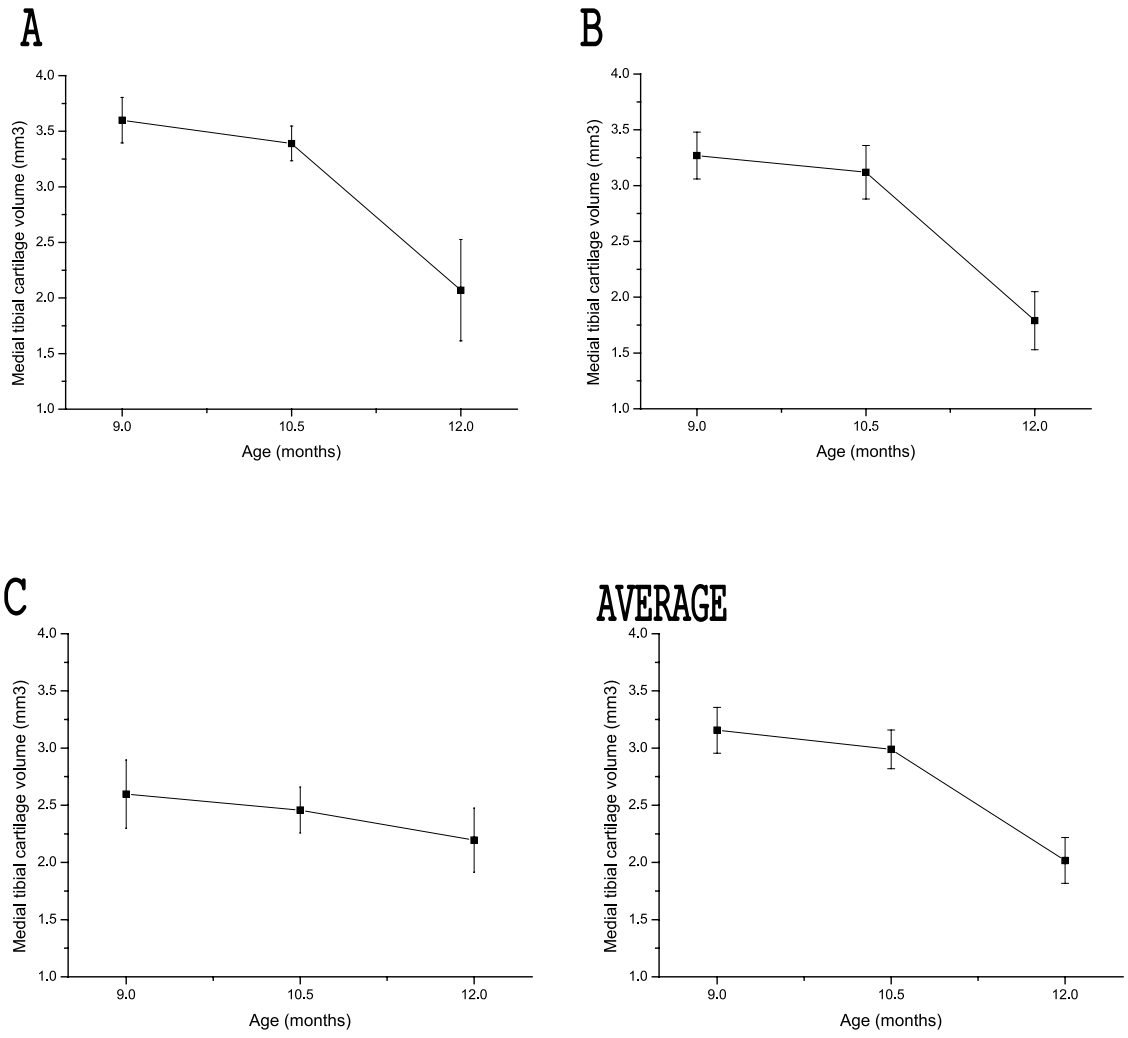

Fig. 4. Volume of the medial tibial cartilage (mean of seven animals±S.E.M.) measured by three observers (Panels A, B, C) at 9, 10.5 and 12 months. On average (Panel D), 5% of the cartilage was lost in 1.5 months and 36% in 3 months.

main source of variability in the segmentation was inter-animal (coefficient of variation=41.8%) and inter-observer (coefficient of variation=34.8%). The intra-observer coefficient of variation was 5%.

Over the 9-month period of the study, the weight of the animals increased from 550 g to 1.2 kg on average (Table I). The limited inter-animal weight variability was not sufficient for us to identify a correlation between animal weight at 12 months and cartilage loss (P=0.14).

Discussion

Imaging obtained in this study shows the various facets of the anatomical changes associated with OA, as previously reported by Watson *et al.*[21] using 2D MRI. We have developed the methodology further and shown for the first time the ability of high-resolution *in vivo* 3D MRI of the guinea pig knee to characterise OA progression. Despite the high resolution, the acquisition time (105 min) was suitable for *in vivo* studies and therefore permitted longitudinal characterisation of OA over the entire guinea pig knee joint. Early indications of disease were apparent at 3 months with cartilage swelling on the medial tibial plateau and meniscal degeneration. By 6 months, small epiphyseal cysts developed, becoming larger at 9 months. At this age, fragmentation and holes in the medial tibial cartilage were clearly seen on the MR images. This was consistent with the progression of OA in this model[7–9] which is known to affect the medial side preferentially. At 12 months, severe fragmentation of the medial tibial cartilage led to localised cartilage thinning. Between 3 and 12 months, there was a progressive loss of MR signal from the posterior meniscus. Histology confirmed this to be meniscal ossification[30].

The changes in joint pathology characterised using MRI are consistent with that observed histologically by us and also reported in the literature for this animal model[4]. However, 3D histological assessment is invasive, time consuming and technically challenging. Therefore in order to compare our 3D MR images with an independent evaluation of cartilage loss, we developed a macroscopic assessment based on staining of articular cartilage with India Ink[29] to visualise areas of cartilage damage. This technique allowed us to characterise cartilage degeneration ranging from mild surface pitting to full thickness cartilage loss. The changes seen by MRI were consistent

with the loss of cartilage visualised using this macroscopic technique.

The quality of the MR images was sufficient to permit blinded manual segmentation of the medial tibial plateau cartilage to give a volumetric measurement. There was a small 5% decrease of cartilage volume between 9 and 10.5 months. The loss of cartilage became considerably larger as the disease progresses, and on average 36% of the medial tibial plateau cartilage was lost from 9 to 12 months. The main source of variability in the cartilage segmentation was inter-animal (41.8%) and inter-observer (34.8%). The high inter-observer variability indicates that to improve the study, all animals in any one study should be segmented by one observer.

At the time of the study no independent method of measuring cartilage volume was available to us, however, in future studies this should be addressed. In addition, formal assessment of the imaging procedure error would further improve our overall confidence in volumetric results. This assessment could be done through the acquisition of multiple data sets in several animals and by analysing the precision of all post-processing steps. Further work is also ongoing to enhance the segmentation protocol and further reduce measurement variability.

Bendele and Hulman[6] reported that OA progression was body weight dependent, however, in this study, the limited inter-animal weight variability was not sufficient for us to identify a correlation between animal weight and cartilage loss.

The use of 3D MR imaging is gaining wider acceptance over conventional joint-space narrowing measurements by X-ray for human articular joint studies. Quantitative monitoring of disease progression by MRI in the clinic is particularly powerful when associated with cartilage segmentation and volumetric measurements[10–14]. The guinea pig knee study presented here shows for the first time that 3D MRI can also be used to examine small animal joints *in vivo* with sufficient resolution to permit cartilage segmentation. Taking into consideration the life span of a guinea pig (~3 years), we can hypothesise that the observed cartilage loss in this guinea pig knee study (~35% in 3 months) is comparable to the rate of knee cartilage loss in human OA patients (~5% per year[14]).

The use of non-invasive 3D *in vivo* MRI to measure cartilage volume changes in small animal models of OA that we have developed is particularly advantageous because for the first time, it does permit comparative efficacy studies between pre-clinical (animal models) and clinical using the same methodology.

References

1. Schwartz ER, Greenwald RA. Experimental models of osteoarthritis. Bull Rheum Dis 1979;30:1030–3.
2. Schwartz ER. Animal models: a means to study the pathogenesis of osteoarthritis. J Rheumatol 1987; 14:101–3.
3. Brandt KD. Animal models of osteoarthritis. Biorheology 2002;39:221–35.
4. Bendele AM, Hulman JF. Spontaneous cartilage degeneration in guinea pigs. Arthritis Rheum 1988; 31:561–5.
5. Wei L, De Bri E, Lundberg A, Svensson O. Mechanical load and primary guinea pig osteoarthrosis. Acta Orthop Scand 1998;69:351–7.
6. Bendele AM, Hulman JF. Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea pigs. Arthritis Rheum 1991;34:1180–4.
7. Hedlund H, De Bri E, Mengarelli-Widholm S, Reinholt FP, Svensson O. Ultrastructural changes in primary guinea pig osteoarthritis with special reference to collagen. APMIS 1996;104:374–82.
8. Wei L, Svensson O, Hjerpe A. Proteoglycan turnover during development of spontaneous osteoarthrosis in guinea pigs. Osteoarthritis Cartilage 1998;6:410–6.
9. Wei L, Svensson O, Hjerpe A. Correlation of morphologic and biochemical changes in the natural history of spontaneous osteoarthrosis in guinea pigs. Arthritis Rheum 1997;40:2075–83.
10. Gandy SJ, Dieppe PA, Keen MC, Maciewicz RA, Watt I, Waterton JC. No loss of cartilage volume over three years in patients with knee osteoarthritis as assessed by magnetic resonance imaging. Osteoarthritis Cartilage 2002;10:929–37.
11. Beaudoin G, Kauffmann C, Godbout B, Raynauld JP, Berthiaume MJ, de Guise J, *et al.* Knee osteoarthritis progression evaluated by magnetic resonance imaging and a novel quantification software tool. Proceedings of the International Society of Magnetic Resonance in Medicine 9th Scientific Meeting and Exhibition, Glasgow, UK, 21–24 April, 2001. p. 80.
12. Peterfy C, White D, Zhao J, VanDijke C, Genant HK. Longitudinal measurement of knee cartilage volume in osteoarthritis (abstract). Arthritis Rheum 1998;41: S361.
13. Glaser C, Draeger M, Englmeier KH, Eckstein F, Reiser M. Cartilage loss over two years in femorotibial osteoarthritis. Radiology 2002;225:330.
14. Wluka AE, Stuckey S, Snaddon J, Cicuttini FM. The determinants of change in tibial cartilage volume in osteoarthritic knees. Arthritis Rheum 2002;46: 2065–72.
15. Altman R. Design and conduct of clinical trials in patients with osteoarthritis. Osteoarthritis Cartilage 1996;4:217–43.
16. Dougados M, Nguyen M, Berdah L, Mazieres B, Vignon E, Lequesne M. Evaluation of the structure-modifying effects of diacerein in hip osteoarthritis: ECHODIAH, a three-year, placebo-controlled trial. Evaluation of the chondromodulating effect of diacerein in OA of the hip. Arthritis Rheum 2001;44: 2539–47.
17. Reginster JY, Deroisy R, Rovati LC, Lee RL, Lejeune E, Bruyere O, *et al.* Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. Lancet 2001;357: 251–6.
18. Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis. A 3 year, randomized, placebo-controlled, double blind study. Arch Intern Med 2002;162:2113–23.
19. Munasinghe JP, Tyler JA, Carpenter TA, Hall LD. High resolution MR imaging of joint degeneration in the knee of the STR/ORT mouse. Magn Reson Imaging 1995;13:421–8.
20. Loeuille D, Gonord P, Guingamp C, Gillet P, Blum A, Sauzade M, *et al. In vitro* magnetic resonance microimaging of experimental osteoarthritis in the rat knee joint. J Rheumatol 1997;24:133–9.

21. Watson PJ, Carpenter TA, Hall LD, Tyler JA. MR protocols for imaging the guinea pig knee. Magn Reson Imaging 1997;15:957–70.
22. Calvo E, Palacios I, Delgado E, Ruiz-Cabello J, Hernandez P, Sanchez-Pernaute O, *et al.* High-resolution MRI detects cartilage swelling at the early stages of experimental osteoarthritis. Osteoarthritis Cartilage 2001;9:463–72.
23. Gahunia HK, Babyn P, Lemaire C, Kessler MJ, Pritzker KPH. Osteoarthritis staging: comparison between magnetic resonance imaging, gross pathology and histopathology in the rhesus macaque. Osteoarthritis Cartilage 1995;3:169–80.
24. Laurent D, Wasvary J, Yin J, Rudin M, Pellas TC, O'Byrne E. Quantitative and qualitative assessment of articular cartilage in the goat knee with magnetization transfer imaging. Magn Reson Imaging 2001; 19:1279–86.
25. Nolte-Ernstring CCA, Adam G, Buhne M, Prescher A, Gunther RW. MRI of degenerative bone marrow lesions in experimental osteoarthritis of canine knee joints. Skeletal Radiol 1996;25:413–20.
26. Bendele AM, White SL, Hulman JF. Osteoarthrosis in guinea pigs: histopathologic and scanning electron microscopic features. Lab Anim Sci 1989;39:115–21.
27. Jimenez PA, Glasson SS, Trubetskoy OV, Haimes HB. Spontaneous osteoarthritis in Dunkin Hartley guinea pigs: histologic, radiologic, and biochemical changes. Lab Anim Sci 1997;47:598–601.
28. Akima H. A method for bivariate interpolation and smooth surface fitting for irregularly distributed data points. ACM Trans Math Softw 1978;4:148–59.
29. Richardson CD, Bae WC, Fazeli B, Filvaroff EH, Sah RL. Quantitative characterisation of osteoarthritis in the guinea pig. ORS Trans, 42nd Annual Meeting, San Francisco, USA, 25–28 February 2001;26.
30. Kapadia RD, Badger AM, Levin JM, Swift B, Bhattacharyya A, Dodds RA, *et al.* Meniscal ossification in spontaneous osteoarthritis in the guinea-pig. Osteoarthritis Cartilage 2000;8:374–7.