Subchondral bone remodeling increases in early experimental osteoarthrosis in young beagle dogs

Harri E Panula[1], Jyrki Nieminen[2], Jyrki J Parkkinen[3], Ilkka Arnala[4], Heikki Kröger[1] and Esko Alhava[1]

We evaluated subchondral bone remodeling and structure in the condyles of the femur and the patellar surface of the femur in early experimental osteoarthrosis of young female beagle dogs.

14 littermate (twin) dogs were divided into operation (n 7) and control groups (n 7). The dogs in the operation group underwent surgically a 30° valgus angulation of the right tibia to induce osteoarthrotic articular cartilage lesions in the knee (stifle) joint. 7 months postoperatively, bone samples were harvested from both condyles and the patellar surface of the femur and evaluated by histomorphometry of subchondral bone. Cartilage samples from the same areas were taken for histology.

In the operated dogs, subchondral bone remodeling increased strikingly in the patellar surface of the femur; osteoid thickness and osteoblast surface/bone surface increased up to 42% and 94% ($p < 0.05$), as compared to controls. Total and active erosion depths increased by 14% and 30% in the same area ($p < 0.05$). However, in bone structural parameters no significant difference could be observed between the groups. In the medial condyle of the femur, the trabecular number decreased in operated dogs, as compared to controls ($p < 0.05$). The lateral condyle of the femur in operated animals did not differ from controls in the parameters tested. In the operated dogs, histology from cartilage samples showed initial osteoarthrotic changes in the patellar surface and the medial condyle of the femur. Histologic changes were greatest in the patellar surface of the femur, as assessed by the Mankin scores.

At the very onset of osteoarthrosis, subchondral bone remodeling increases, but the bone structural changes are indistinct. It seems that in this osteoarthrosis model, cartilage lesions precede major subchondral changes in the structure of the bone.

Departments of [1]Surgery and [3]Pathology, Kuopio University Hospital, [2]Surgery, Mikkeli Central Hospital, [4]Surgery, Hyvinkää District Hospital, Finland. Correspondence: Dr. Harri E. Panula, Neulaskuja 1, FI-60510 Hyllykallio, Finland
Tel +358 17-163000. Fax -63032
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Different parts of the joint, bones, articular cartilage, synovial membrane and synovial fluid form an interactively functioning unit. Sokoloff (1969) has emphasized that in osteoarthrosis (OA) all the structures of the joint are affected to some extent because of their interrelationship. It is not known what is the primary cause leading to OA, certainly, mechanical and constitutional factors play a role. Some authors believe that the primary lesion occurs in cartilage (Bollet 1969, Lee et al. 1974), the subchondral bone (Radin 1973, 1976), or synovium (Glynn 1977). However, any of these could be the primary event in different pathophysiological pathways to OA. Animal models have provided valuable insight into the disease process. These include the use of mice strains having natural OA (Sokoloff 1956, Benske et al. 1988), and canine, rabbit and guinea pig models subjected to altered joint mechanics or various joint loading conditions (Radin et al. 1973, 1984, Reimann 1973, Layton et al. 1988, Wu et al. 1990, Brandt et al. 1991, Dedrick et al. 1993).

Recently, we have reported a slowly progressive OA model in young beagle dogs, utilizing high tibial 30° valgus osteotomy (Panula et al. 1997). 7 months after surgery, we found very initial microscopic articular cartilage changes in the knee (stifle) joint of the operated hindlimb. During the following 11 months, macroscopic osteoarthrotic cartilage lesions became evident. In operated animals, the histologic changes (i.e., Mankin scores) exceeded the control level ($p < 0.05$).

In the present study, we focused our interest on the subchondral bone of both condyles and the patellar surface of the femur in the same young beagle dogs showing very early signs of articular cartilage deterioration leading later to manifest OA. We utilized bone histomorphometry to evaluate bone turnover and structure.

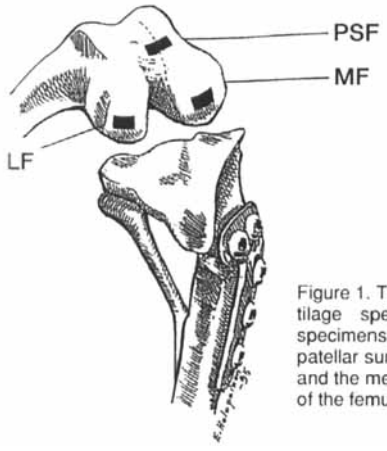

Figure 1. The sites of bone and cartilage specimen collection. The specimens were harvested from the patellar surface of the femur (PSF), and the medial and lateral condyles of the femur (MF, LF).

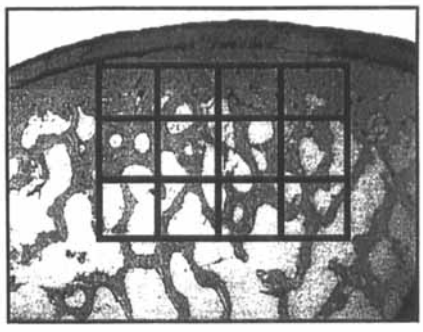

Figure 2. Representation of the analysis field for histomorphometry on a bone section (5 μm thick) from the lateral condyle of femur.

Animals and methods

Animal preparation

The animals were the same as in our previous study (Panula et al. 1997). 14 female beagle dogs of pure breed and same age were purchased from Marshall Farms (North Rose, NY, USA). The dogs formed 7 pairs, each of which were twins. The animals were divided into operated (n 7) and control (n 7) groups. A slowly progressive OA was induced in the right knee (stifle) joint of the dogs by a method described earlier (Panula et al. 1997). Briefly, for 7 dogs a 30° valgus angulation to the right tibia was created by operation at the age of 3 months. The osteotomized bones were internally fixed with plates. 7 dogs without operation were littermate controls. The dogs were fed with commercial dog food (Vaasan Mylly Oy, Vaasa, Finland) 240 g per day. Water was given ad libitum. During the experiment, the dogs were kept under kennel conditions in the National Laboratory Animal Center (Karttula, Finland), where they were housed in standard cages with a bottom area of 0.9 × 1.2 m and height of 0.8 m. The Animal Care and Use Committee of the University of Kuopio approved the experimental design. The experimental protocol complied with the principles of the Care and Use of Animals (NIH publication 1985).

The animals were killed by an overdose of anesthetic 7 months after operation. Their weights were recorded. The hindlimbs were dissected free from the muscles and the knee was opened. Bone blocks 5 mm × 10 mm × 5 mm in size were sawn off from the centromedial part of the patellar surface of the femur and both condyles (center) of the femur (Figure 1). After sawing, the blocks were immediately transferred to absolute ethanol, which was changed 4 times during the first week. 1 mm thick cartilage slices for histologic grading of cartilage lesions were cut perpendicularly to the cartilage surface with underlying subchondral bone beside the bone blocks taken for bone histomorphometry (Panula et al. 1997).

Subchondral bone analysis

After fixation in absolute ethanol, bone samples were dehydrated and embedded in methylmethacrylate. 5 μm sections were cut, using a Reichert-Jung K polycut S microtome with HK knives (Jung, Heidelberg, Germany). After dissolving methylmethacrylate, the sections were stained using the modified Masson-Goldner trichrome staining. Histomorphometric analysis of bone sections was performed using a semi-automatic image analysis system (Osteoplan II) (Zeiss, Thornwood, NY, USA) with magnification of × 200 (Malluche et al. 1982). The measurements were performed by counting bone cells and tracing lengths of individual features and circuiting area.

The following histomorphometric parameters of bone were evaluated: trabecular bone volume (BV/total volume), trabecular thickness, trabecular number, osteoid volume (OV/BV), osteoid surface/bone surface (OS/BS), osteoid thickness, osteoblast number/bone perimeter (B.Pm), osteoblast surface (ObS/BS), total eroded surface (ES/BS), active eroded surface (ES[osteoclast+]/BS), total erosion depth, active erosion depth (osteoclast+), osteoclast number (/B.Pm) and osteoclast surface (OcS/BS) (Parfitt 1988).

The measurements were performed on subchondral bone plate of operated and control dogs. The upper side of the measured rectangle was adjusted just below the tidemark of articular cartilage. Measured depth was 186 μm and width 248 μm. Further, this area was divided into 12 regions of equal size, on which the histomorphometry was done (Figure 2). The final result concerning the parameter of interest was calculated as a mean (SEM) value in these 12 regions.

Table 1. Bone histomorphometry from the right femur condyles and patellar surface of femur in young female beagle dogs 7 months after 30° high tibial valgus osteotomy (n 7) and in controls (n 7), values are mean (SEM) and change in % (Δ%)

	Patellar surface			Lateral condyle			Medial condyle		
	Control	Osteotomy	Δ%	Control	Osteotomy	Δ%	Control	Osteotomy	Δ%
Bone structure									
Trabecular bone volume (%)	44 (1.9)	46 (1.5)	3	51 (1.8)	49 (2.0)	–4	46 (3.3)	43 (2.2)	–5
Trabecular thickness (μm)	96 (5.5)	103 (4.7)	7	116 (3.3)	116 (4.1)	~0	91 (5.7)	97 (5.4)	6
Trabecular number	4.7 (0.1)	4.5 (0.1)	–4	3.4 (0.1)	3.3 (0.1)	–3	5.0 (0.2)	4.5 [a] (01.)	–10
Bone formation									
Osteoid volume (%)	0.8 (0.2)	1.2 (0.1)	50	0.5 (0.1)	0.6 (0.1)	20	0.6 (0.1)	0.6 (0.2)	~0
Osteoid surface (%)	7.9 (1.4)	9.8 (0.6)	24	6.2 (1.5)	7.1 (1.1)	15	5.1 (1.0)	6.3 (18)	24
Osteoid thickness (μm)	4.5 (0.5)	6.4 [a] (0.3)	42	4.7 (0.3)	4.7 (0.5)	0	5.6 (0.5)	4.6 (0.6)	–18
Osteoblast number	124 (59)	163 (42)	32	110 (26)	183 (53)	66	110 (31)	127 (48)	16
Osteobl. surface (mm^2/cm^3)	1.8 (0.6)	3.5 [a] (0.6)	94	1.8 (0.5)	2.8 (0.8)	56	1.4 (0.4)	1.6 (0.6)	14
Bone erosion									
Total eroded surface (%)	7.4 (0.9)	7.5 (1.4)	1	7.7 (1.0)	8.1 (1.3)	5	7.7 (0.4)	7.7 (0.5)	~0
Active eroded surface (%)	2.3 (1.9)	2.4 (1.2)	4	1.1 (0.8)	1.9 (1.5)	73	1.1 (0.5)	0.7 (0.6)	–36
Total erosion depth (μm)	3.7 (0.2)	4.2 [a] (0.3)	14	3.9 (0.3)	4.1 (0.2)	5	3.2 (0.2)	3.4 (0.2)	6
Active erosion depth (μm)	4.4 (1.1)	5.7 [a] (1.2)	30	5.3 (2.2)	4.4 (1.5)	–17	3.4 (1.6)	4.0 (2.2)	18
Osteclast number	38 (13)	35 (5.2)	–7	17 (4.6)	33 (8.2)	99	16 (0.3)	14 (5.4)	–14
Osteclast surface (%)	1.4 (0.5)	1.6 (0.3)	14	0.7 (0.2)	1.4 (0.4)	100	0.7 (0.2)	0.6 (0.2)	–14

[a] $p < 0.05$, versus control, Wilcoxon matched-pairs signed-ranks test

Cartilage specimens

The procedure to prepare articular cartilage sections for histologic grading of cartilage lesions has been previously described (Panula et al. 1997). Briefly, the sections were stained with Weigert's iron hematoxylin, safranin-O and fast green. The severity of cartilage lesions was assessed by the scoring system presented by Mankin et al. (1971).

The thickness of articular cartilage was determined with the computerized image-analysis system for polarized light microscopy (Arokoski et al. 1996). It consisted of a Leitz Ortholux® II POL polarized light microscope (Leitz Wetzlar, Wetzlar, Germany), connected to a thermoelectrically-cooled camera Photometrics CH250/A (Photometrics Inc., Tucson, AZ, USA), including the Kodak KAF-1400 CCD (Kodak, Rochester, NY, USA). The beginning of the superficial zone and the tidemark were digitally marked. Based on these markings, the system calculated the mean cartilage thickness.

Statistical analysis

The Wilcoxon matched-pairs signed-ranks test was used to calculate statistical significance of differences between the operated and control dogs. A p-value less than 0.05 was considered significant. For correlations, Pearson's linear correlation analysis was used.

Results

Body weight

The mean weight of operated dogs was 9 (7.6–10.6) kg and of controls 8.8 (7–11.5) kg.

Histomorphometry of subchondral bone (Table 1)

The differences between bone structure parameters did not reach statistical significance between operated and control dogs, except for trabecular number in the medial condyle of the femur, where the number decreased in operated animals, as compared to controls ($p < 0.05$). This was associated with a ($p = 0.7$) decreased trabecular bone volume that was not significant. In the patellar surface of femur osteoid thickness, osteoblast surface, erosion depth and active erosion depth, as indicators of bone remodeling, increased in operated dogs more than in controls ($p < 0.05$). Increases in osteoid volume and osteoid surface did not reach statistical significance ($p = 0.2$).

Histology of articular cartilage

Cartilage pathology is given in detail in our previous study (Panula et al. 1997). Briefly, histologic changes exceeded the age-matched control level ($p < 0.05$), as assessed by the scoring system presented earlier (Mankin et al. 1971, Panula et al. 1997). In the operated dogs, microscopic cartilage changes were found on the patellar surface and medial condyle of the femur. Histologic changes were greatest in the patellar surface of the femur. Samples from control animals showed normal articular histology.

Table 2. Correlation [a] of femoral bone structure, formation and erosion with histologic changes (Mankin scores) in the knee (stifle) joint 7 months after 30° high tibial valgus osteotomy in young female beagle dogs

	Patellar surface		Lateral condyle		Medial condyle	
	r	p-value	r	p-value	r	p-value
Bone structure						
Trabecular bone volume (%)	0.11	0.09	0.37	0.2	–0.08	0.8
Trabecular thickness (µm)	0.26	0.4	0.29	0.3	–0.35	0.2
Trabecular number	–0.18	0.09	0.12	0.7	–0.06	0.8
Bone formation						
Osteoid volume (%)	0.45	0.1	0.37	0.2	0.19	0.5
Osteoid surface (%)	0.61	0.03	0.28	0.3	–0.08	0.8
Osteoid thickness (µm)	0.48	0.1	0.04	0.9	0.20	0.5
Osteoblast number	0.24	0.4	0.52	0.06	–0.14	0.6
Osteobl. surface (mm^2/cm^3)	0.49	0.09	0.42	0.1	–0.13	0.7
Bone erosion						
Total eroded surface (%)	0.07	0.8	–0.10	0.7	–0.15	0.6
Total erosion depth (µm)	0.66	0.01	0.13	0.7	–0.43	0.1
Active erosion depth (µm)	0.42	0.2	0.21	0.5	–0.24	0.4
Osteclast number	0.11	0.7	0.31	0.3	0.03	0.9
Osteclast surface (%)	0.20	0.5	0.36	0.2	0.05	0.9

[a] Pearson's correlation test

The mean thickness (mm) of articular cartilage in operated dogs was 0.53, 0.66 and 0.83 in the patellar surface of the femur and lateral and medial condyles of the femur, respectively. The corresponding values for controls were 0.46, 0.58 and 0.83. In the lateral condyle of the femur, the difference was statistically significant ($p = 0.04$).

Relationship between bone and cartilage changes (Table 2)

In the patellar surface of the femur (valgus-operated dogs), the severity of cartilage lesions (i.e., Mankin scores) correlated significantly with erosion depth and osteoid surface (r-values 0.66 ($p < 0.05$) and 0.61 ($p < 0.05$), respectively).

Discussion

Our study was designed to investigate microscopic bone changes in a skeletally immature canine model of very early OA. We focused our interest on the condyles and patellar surface of the femur to evaluate the effects of altered mechanical forces on the structure and turnover of bone at these sites. The tibial condyles were not included because of the proximity of the tibial osteotomy which could have confounded interpretation of the results, due to the regional acceleratory phenomenon (RAP) described by Frost (1983). We found a profound increase in subchondral remodeling in operated dogs as compared to control dogs. The changes were most obvious in osteoid-related parameters, particularly in the patellar surface of the femur, but there was also an increase in erosion depth in the same area. We have earlier shown that in this model, 7 months after surgery, initial microscopic articular cartilage changes progressed to lesions, typical of OA, 18 months after the operation (Panula et al. 1997). We concluded that the valgus osteotomy induced OA changes because of the altered loading pattern of the knee joint. Moreover, the valgus osteotomy did not merely shift joint loading towards the lateral compartment of knee joint. It also shifted the patellar pull medially altering the patellofemoral articulation. At the same time, the knee remained entirely stable (Panula et al. 1997).

In interpreting the results of bone histomorphometry, it is important to take into account the population of animals used in the study. The experimental animals in our study were very homogeneous, i.e., the operated and control dogs were twins, and of the same gender, and all the 7 twins were of equal age. The body weights of animals did not differ. We also studied the gait pattern and weight bearing of the limbs of the operated and control dogs after the operation, by using an electrothermomechanical film to register the forces under the paws. 3 months after the valgus operation, no differences in weight bearing of the limbs could be detected between operated and control dogs (Heikkinen et al. 1997). We also believe that the RAP does not influence the metabolism of bone in the femur. In the light of these considerations, we regard the results as very reliable, despite the small number of animals in this study.

The ability of 2-dimensional (2-D) plate model-based bone histomorphometry to detect subtle structural alterations is limited. Although there were no statistically significant changes in trabecular bone volume between the groups, we cannot exclude the possibility that changes in the subchondral bone microarchitecture have occurred in our samples. In fact, there was a 10% decrease in trabecular number in the medial femoral condyle that was associated with a decrease in bone volume fraction that was not significant. There were similar but insignificant changes in the lateral femoral condyle. Tb.N is an indirect measurement, based on the plate model and derived from the ratio between trabecular surface and bone volume fraction. Thus, the findings above could indirectly indicate a decrease in trabecular surface and thickening of the subchondral bone plate in our model. Unfortunately, direct measurement of the thickness of subchondral bone plate is difficult and subject to many errors. Recent development in 3-dimensional (3-D) imaging is promising for assessing 3-D structure and mechanics of cancellous bone (Odgaard 1997).

There are suggestions that primary OA could initially be a bone disease rather than a cartilage disease. Little attention has been paid to changes in the subchondral bone, because they have been considered to be secondary. Johnsson (1962) has proposed that bone remodeling could lead to degeneration of cartilage. Radin et al. have worked to formulate a hypothesis that one of the mechanisms triggering cartilage degeneration may be a steep stiffness gradient in the underlying subchondral bone (Radin et al. 1973, 1984, Radin and Rose 1986). It is thought that repeated failure of the musculoskeletal system around the joint to attenuate peak dynamic forces (repetitive impulse loading) cause the subchondral stiffening. Thus, the stiffened bone no longer acts as an effective shock absorber, exposing the overlying articular cartilage to greater peak dynamic forces and, moreover, later to cartilage damage. According to Radin et al., it is the healing of trabecular microfractures that leads to stiffening of subchondral bone. In our study, mechanical testing of cancellous bone might also provide valuable information in the future.

Dequeker et al. (1993) have an approach quite similar to the etiopathogenesis of OA as also have Radin et al., but they consider the stiffness of subchondral bone to be a part of more general bone alteration. They found an association between OA and skeletal content of different growth factors, reflecting a generalized increased biosynthetic activity of osteoblasts in OA (Dequeker et al. 1993).

Our canine OA model induces cartilage changes in two ways. First, the valgus osteotomy shifts joint loading towards the lateral compartment of the joint, making articular cartilage prone to increased loads and damage. Secondly, due to the malalignment of the operated hindlimb, the patellar pull shifts medially, leading to subluxation of the patella over the anterior aspect of medial condyle of femur (Panula et al. 1997). Medial subluxation movement of the patella from its femoral groove induces shear forces which harm the articular cartilage of the patellar surface, and the anterior surface of the medial condyle of the femur, too. In this study, the initial lesions and increase in thickness of the articular cartilage are in agreement with the above-mentioned alterations in mechanics of the knee (stifle) joint after tibial valgus osteotomy. Bone remodeling alterations are also well in line with the changed mechanics in the knee joint reflecting, on the one hand, increased joint loading in the lateral compartment and patellofemoral joint and, on the other hand, decreased joint loading in the medial compartment. It is tempting to suggest that, in contrast to previous studies, in this model, cartilage lesions precede major structural changes (sclerosis) of the subchondral bone, although we cannot totally exclude the probable thickening of the subchondral bone plate. Hence, it is too simple to consider OA only as a disease of articular cartilage. On the contrary, the changes found in bone remodeling and subtle changes in structure suggest that subchondral bone (trabecular) thickening may occur later in the course of the disease. Further studies (i.e., mechanical testing, 3-D imaging) are needed to confirm that this occurs.

In summary, in this experimentally-induced canine OA model, articular cartilage changes precede structural alterations in subchondral bone. However, signs of altered bone remodeling occur concomitantly with articular cartilage lesions.

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