The Effect of Varus Stress on the Moving Rabbit Knee Joint

KOSUKE OGATA, M.D., LEO A. WHITESIDE, M.D., PEGGY A. LESKER, B.S. AND DAVID J. SIMMONS, PH.D.

Many attempts have been made to induce osteoarthritis in experimental animals by altering mechanical forces across the joints. This has been accomplished by immobilization,[6, 12, 13, 24, 25] compression,[8, 11, 21, 23] and excision of the medial collateral and cruciate ligaments.[1, 2, 18] Results using these methods seem to mimic the course of degenerative joint disease caused by abnormal biomechanical conditions such as cast immobilization with forced position, joint instability or deformity. The animal models do not, however, produce an arthritic pattern which is entirely analogous to the situation in man. In the human knee joint, the osteoarthritic changes are usually focal in character and are first confined to one of the femoral condyles or tibial plateaus,[3, 14] whereas the existing animal models cause damage in both compartments of the knee. An attempt was made in the present investigation to produce a mechanical derangement which would cause a gradually progressive unicompartmental osteoarthritis without opening or immobilizing the knee. This paper presents our technique for producing varus stress in non-immobilized rabbit knees and describes the morphological changes which occur with this model.

From the Division of Orthopedic Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, Missouri 63110.

Received: April 8, 1977.

MATERIALS AND METHODS

New Zealand white rabbits weighing 1.82–3.40 kg were used as experimental animals. Under intravenous pentobarbital anesthesia, threaded Kirschner wires were placed transversely through the distal metaphyses of the right femurs and the proximal metaphyses of the right tibiae approximately at 1.5 cm from the joint line using aseptic technique. The wires were left protruding through the medial side and were connected by springs adjusted to apply varus stress to the knee joints. The distance between the Kirschner wires and the length of the springs were measured with the knees in full flexion, 90° flexion and full extension. A standard stress-strain curve was made for the springs using a tensiometer and the tension was adjusted so that with the knees in 90° flexion, the varus stress ranged between 350 and 1150 grams. Most rabbit knees were subjected to forces between 700 and 900 g. The tension of the spring was minimal with the knee in full flexion, ranging between zero and 300 g. The left knees were used as controls and threaded wires of the same size were placed in the distal femurs and proximal tibiae, but springs were not applied (Fig. 1). After the operative procedure, the rabbits were observed to use their operated extremities for normal activity in their

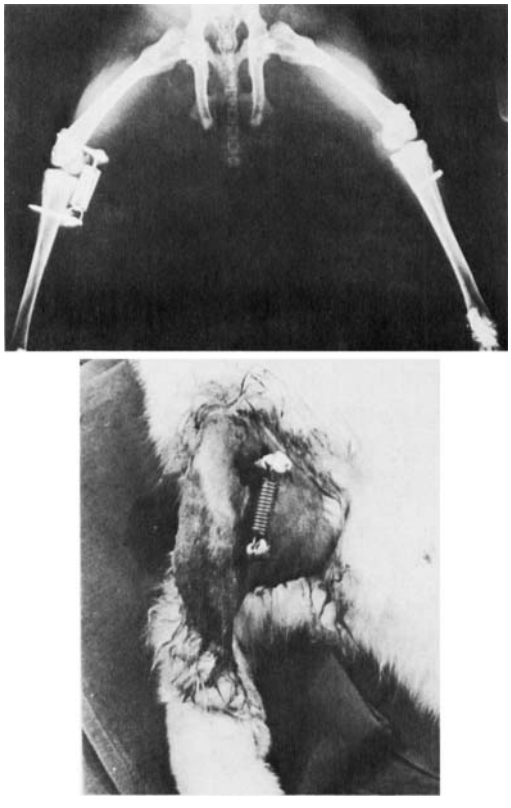

FIG. 1. Postoperative roentgenograms of the rabbit knee joints and photograph of anterior aspect of the right knee with varus stress applied.

cages. The animals were divided into 6 groups of 8 rabbits each which were examined at intervals of 1, 2, 3, 4, 6, and 8 weeks after the application of the springs.

At necropsy, the tensions of the springs were recalibrated; there was generally a slight loss of tension ($= \sim 10\%$). Internal rotation of the right legs ($15 \sim 30°$) was noticed in almost all animals. Each knee joint was carefully inspected and the lesions of the articular cartilage of the proximal tibiae were graded grossly as 0, I, II and III using the classification system of Salter and Field:[21] Grade 0–normal; Grade I–loss of normal luster and translucency with yellowish discoloration of the cartilage and increased softness to touch; Grade II–partial-thickness loss of cartilage or blister formation in center of lesion with Grade I change in the periphery; Grade III–loss of full thickness of cartilage, exposing subchondral bone in the center of the lesion with less severe changes in the periphery.

Two or 3 rabbits in each group were selected at random for microscopic studies. The proximal and distal thirds of the tibia and femur respectively were fixed in 10% neutral formalin, decalcified in a formic acid-citric acid solution and processed by routine procedures to obtain paraffin section. Serial sections cut parallel to the long axis of the bone were stained with hematoxylin-eosin, alcian blue in 0.4–0.5M M_gCl_2, toluidine blue and safranin-O. The rest of the rabbits were used for biochemical studies of articular cartilage (to be reported separately).

RESULTS

GROSS CHANGE

Gross pathological changes were found only in the medial tibial and the medial femoral articular cartilage of the knees subjected to varus stress (Fig. 2). The obvious lesions were localized in the center of the tibial condyle not covered by the meniscus and in the posterior portion of the medial femoral condyle. No gross lesions were seen in the lateral compartments of the knees nor in the patello–femoral joints. The left knees which were used as controls were unremarkable. After one or two weeks, the majority of the compressed cartilage showed Grade I change with loss of normal luster and yellowish discoloration. Although the correlation between the length of time the varus stress was applied and the grade of lesion was not perfect, the severity of the lesions generally increased with the duration of the varus stress (Fig. 3). After 4–6 weeks, the majority showed Grade II and III changes; however, no further changes were developed after 8 weeks. There was some variation in severity of the lesions among animals at each time period and even within each grade.

MICROSCOPIC CHANGES

In all groups of animals, the histological changes were confined to the medial compart-

ments of the knee joints with varus stress. The severity of the histological changes appeared to be related to the length of time the varus stress was applied. In the first 2 weeks of varus stress, no remarkable histological changes were seen in the articular cartilage and subchondral bone, except in the center of the weight-bearing area. In these areas some pycnosis of chondrocytes was present and there was a slight reduction in the intensity of matrix staining with toluidine blue, alcian blue and safranin-O. More advanced degenerative changes in the articular cartilage began to appear after 3–4 weeks of varus stress. In the center of the weight-bearing area we observed severe pycnosis, loss of numbers, and poor staining of cells. The cellular changes in the periphery of this center area were characterized by chondrocyte cloning, and the cells and matrix were normal in appearance farther peripherally (Fig. 4). Early fasciculation and fibrillation were present at this stage. In some of the severely affected tibial condyles, there were areas of cyst formation within the articular cartilage. These intrachondral cysts were most prevalent deep in the noncalcified layer immediately adjacent to the tidemark, and were never found in the calcified layer (Fig. 5). They did, however, frequently extend into the more superficial zones. Intrachondral cysts were not found in the femoral articular cartilage. A marked reduction of staining with safranin-O and alcian blue was seen in the non-calcified layer, suggesting a loss of mucopolysaccharide, and in particular chondroitin sulfate; however, the calcified layer deep to the tidemark appeared to retain its normal histochemical character. No apparent changes were present in the subchondral bone.

At 6–8 weeks, the cell clusters were more numerous, and in addition, the articular cartilage in the center of the compressed area gradually decreased in thickness with an apparent loss of staining of the cell nuclei.

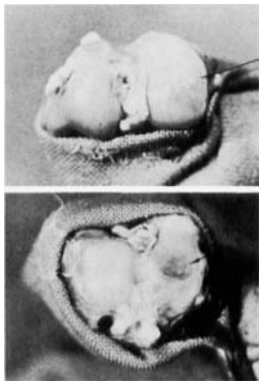

FIG. 2. Gross pathological changes are confined to the medial femoral (top) and tibial (bottom) articular cartilage. Arrows indicate the centers of the lesions.

Below, there were areas of disruption of the tidemark and the calcified layer of the articular cartilage. Increased vasculature of the subchondral bone was found in the areas adjacent to severe cartilage damage, but no remarkable thickening of the bony trabeculae were observed. At this time, in several sections, cartilaginous masses were noted in the adjacent subchondral marrow spaces. These areas of cartilaginous tissue appeared continuous with the damaged areas in the articular cartilage through gaps in the tidemark (Fig. 6).

DISCUSSION

It is generally accepted that human osteoarthritis, in the absence of specific cartilage disease, results from abnormal mechanical

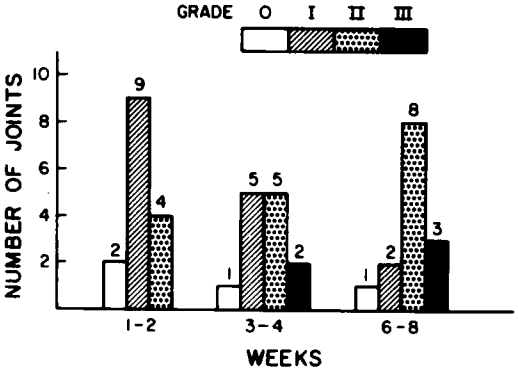

FIG. 3. Gross correlation of severity of lesion versus duration of varus stress.

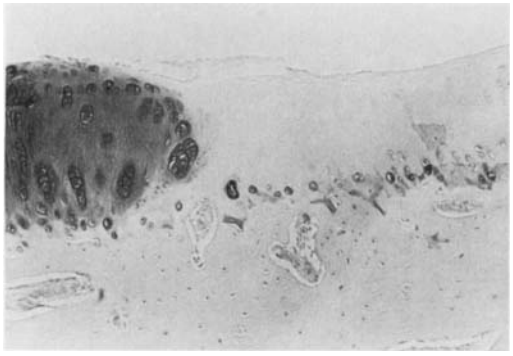

FIG. 4. Articular cartilage of the distal femur shows severe pycnosis, loss of cellularity and a marked reduction in the intensity of matrix staining in the center of the lesion and chondrocyte cloning in the periphery of the lesion. (Safranin–O, × 250)

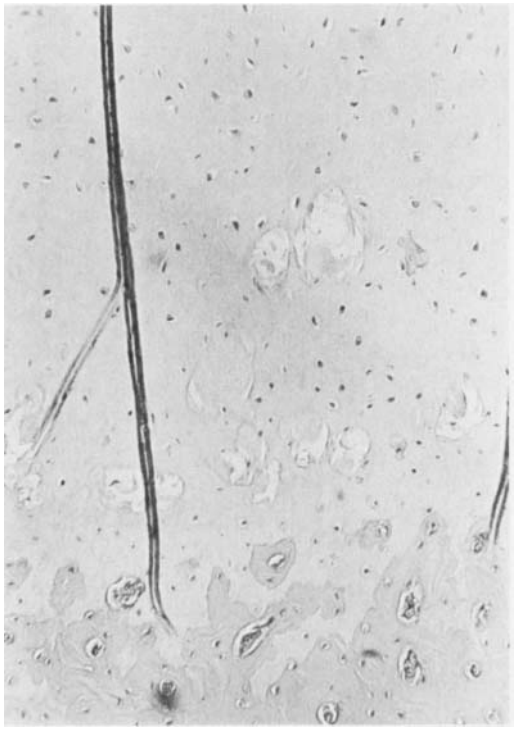

FIG. 5. Deep zones of tibial articular cartilage. Note the intrachondral cysts which are most prevalent deep in the noncalcified layer immediately adjacent to the tidemark (Hematoxylin–eosin, × 450).

forces applied across the joint during normal activity.[4, 7, 16, 22] Our experimental model causes osteoarthritic changes of the medial compartment of the knee which closely mimic this clinical situation. Although the gross and microscopic–histochemical changes were similar to those reported by other investigators using compression or immobilization, our model produced much more gradually progressive lesions. This may be because the joints were allowed to move, thereby facilitating nutrition of articular cartilage,[5, 20] and also because the joints were probably partially unloaded in full flexion.

Our results suggested that, within the range of varus stress used, duration appears to be more important than magnitude of varus stress in determining the severity of cartilage damage. Gritzka *et al.*[11] reached the same conclusion on the basis of rabbit experiments in which continuous compression was applied across the moving elbow joint.

The histological studies presented some remarkable findings. The architecture and safranin-O staining qualities of the calcified cartilage deep to the tidemark were relatively normal, despite advanced degenerative changes in the superficial and middle zones of the articular cartilage. This relative resistance to stress of the calcified zone compared to the more superficial layers may stem from a difference in nutritional pathways.[23] Since the superficial layers may receive their nutrition chiefly through diffusion from synovial fluid and the deep layers may be nourished by diffusion from the subchondral bone, as well as from the synovial fluid,[15] it is possible that the layers deep to the tidemark have some advantage in supply of nutrients during the development of the arthritic process. On the other hand, it is also possible that the mechanical properties of the calcified zone make it more resistant to shear and compressive forces.[10]

In the tibial articular cartilage subjected to abnormal stress, fasciculation and degenerative cysts were frequently found in the middle and deep zones of the noncalcified cartilage (especially along the tidemark), while the superficial layers were often spared. Goodfellow and his coworkers[9] have recently described degeneration in the basilar layers of the articular cartilage lining the human patella. As was found in our study, these lesions appeared to be concentrated in areas normally subjected to the highest compressive and shear stresses. The degenerative changes which we have described may, as Goodfellow has suggested, be explained by mechanical considerations. As Redler *et al.*[19] suggested, the superficial layers of articular cartilage may be very compliant, and the layers deep to the tidemark are less compliant; thus a concentration of shear stresses along the tidemark might be expected. The compliant cartilage layers, adjacent to the tidemark then would be expected to develop early degenerative changes as a result of this excessive stress.

Among the rabbits subjected to varus stress for 6–8 weeks, several animals developed foci of highly cellular cartilaginous tissue in the subchondral marrow spaces of the distal femurs. This cartilaginous tissue appeared to be continuous with the overlying degenerated cartilage, but stained deeply with hematoxylin (suggesting a high degree of calcification) and with safranin-O (suggesting high mucopolysaccharide content). The cellular morphology appeared to gradate from nondifferentiated fibrous tissue cells to well-differentiated cartilage cells, suggesting a metaplastic process. These findings are similar to those described in human osteoarthritis in which deeply-placed bits of cartilage arise from cartilaginous metaplasia of marrow cells. It is not possible in this study to determine the fate of these cartilaginous areas. It is conceivable that they may develop into subchondral cysts, or mature into bony tissue and thus lead to subchondral sclerosis.[17] In our animal model, subchondral bone did not show remarkable changes despite advanced degenerative changes in the articular cartilage. This would suggest that the cartilage is highly susceptible to mechanical stress, and develops degenerative changes prior to any subchondral bone thickening. It is reasonable to expect that changes in subchondral bone will occur after a certain period of time since the overlying articular cartilage degenerates and loses its function as a "shock-absorber."

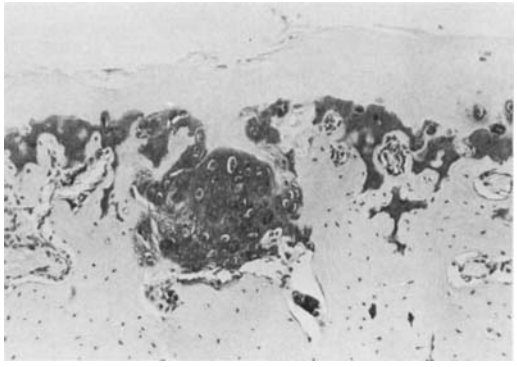

FIG. 6. Cartilaginous mass is noted in the subchondral marrow space which appears continuous with the damaged areas in the articular cartilage. This cartilaginous body and calcified cartilage are stained deeply with safranin—O (× 250).

SUMMARY

Unicompartmental osteoarthritis was produced by applying varus stress to moving rabbit knee joints. Degenerative changes were confined to the medial tibial and the medial femoral articular surfaces. Within the range of varus stress used, duration appears to be more important than magnitude of varus stress in determining the severity of cartilage damage. The calcified zone remained histologically unchanged despite advanced changes in the noncalcified zone superficial to the

tidemark. Intrachondral degenerative cysts were frequently found in the basilar layers of the noncalcified cartilage adjacent to the tidemark where shear stresses were likely to be highest and diffusible nutrients least available. Highly cellular cartilaginous tissue was noted in the subchondral marrow spaces in the specimens with advanced cartilage degeneration. These areas appeared to be continuous with the overlying degenerated cartilage through gaps in the calcified cartilage. Subchondral bone did not show remarkable trabecular thickening despite advanced degenerative changes in the articular cartilage.

REFERENCES

1. Anders, H., Lindberg, L. and Telhag, G.: Experimental osteoarthritis in rabbits. Preliminary report, Acta, Orthop. Scand. 41:522, 1970.
2. Bohr, H.: Experimental osteoarthritis in the rabbit knee joint, Acta. Orthop. Scand. 47: 558, 1976.
3. Coventry, M. B.: Osteotomy of the upper portion of the tibia for degenerative arthritis of the knee. A preliminary report, J. Bone Joint Surg. 47-A:984, 1965.
4. ———: Osteotomy about the knee for degenerative and rheumatoid arthritis: Indication, operative technique and results, J. Bone Joint Surg. 55-A:23, 1973.
5. Ekholm, R.: Nutrition of articular cartilage, Acta. Anat. 24:329, 1955.
6. Evans, B., Eggers, G. W. N., Butler, J. K. and Blumer, J.: Experimental immobilization and remobilization of rat knee joint, J. Bone Joint Surg. 42-A:737, 1960.
7. Frankel, V. H., Burstein, A. H. and Brooks, D. B.: Biomechanics of internal derangement of the knee, J. Bone Joint Surg. 53-A:945, 1971.
8. Ginsberg, J. M., Eyring, E. J. and Curtiss, P. H.: Continuous compression of rabbit articular cartilage producing loss of hydroxyproline before loss of hexosamine, J. Bone Joint Surg. 51-O:467, 1969.
9. Goodfellow, J., Hungerford, D. S. and Woods, C.: Patello-femoral joint mechanics and pathology, J. Bone Joint Surg. 58-B:291, 1976.
10. Green, W. T., Martin, G. N., Eanes, E. D. and Sokolof, L.: Microradiographic study of the calcified layer of articular cartilage, Arch. Pathol. 90:151, 1970.
11. Gritzka, T. L., Fry, L. R. and Cheesman, R. L.: Deterioration of articular cartilage caused by continuous compression in a moving rabbit joint, J. Bone Joint Surg. 55-A: 1698, 1973.
12. Hall, M. C.: Cartilage changes after experimental immobilization of the knee joint of the young rat, J. Bone Joint Surg. 45-A:36, 1963.
13. ———: Articular changes in the knee of the adult rat after prolonged immobilization in extension, Clin. Orthop. 34:184, 1964.
14. Helfet, A.: Disorders of the Knee. Philadelphia, J. B. Lippincott Co., 1974. p. 165.
15. Honner, R. and Thompson, R. C.: The nutritional pathways of articular cartilage, J. Bone Joint Surg. 53-A:742, 1971.
16. Jackson, J. P. and Waugh, W.: Tibial osteotomy for osteoarthritis of the knee, J. Bone Joint Surg. 43-B:746, 1961.
17. Jaffee, H. L.: Metabolic, Degenerative and Inflammatory Diseases of Bones and Joints, Philadelphia, Lea and Febiger, 1972. p.751.
18. McDevitt, C. A .and Muir, H.: Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog, J. Bone Joint Surg. 58-B:94, 1976.
19. Redler, I., Mow, V. C., Zimmy, M. L. and Mansell, J.: The ultrastructure and biomechanical significance of the tidemark of articular cartilage, Clin. Orthop. 112:357, 1975.
20. Saaf, J.: Effects of exercise on adult articular cartilage, Acta. Orthop. Scand. Supp. #7:1, 1950.
21. Salter, R. B. and Field, P.: The effects of continuous compression on living articular cartilage. An experimental investigation, J. Bone Joint Surg. 42-A:31, 1960.
22. Solomon, L.: Patterns of osteoarthritis of the hip, J. Bone Joint Surg. 58-B:176, 1976.
23. Thompson, R. C. Jr. and Bassett, A. L.: Histological observation on experimentally induced degeneration of articular cartilage, J. Bone Joint Surg. 52-A:435, 1970.
24. Troyer, H.: The effect of short-term immobilization on the rabbit knee joint cartilage. A histochemical study, Clin. Orthop. 107:249, 1975.
25. Videman, T., Michelsson, J. E., Rauhamaki, R. and Langenskiold, A.: Changes in ^{35}S-sulphate uptake in different tissues in the knee and hip regions of rabbits during immobilization, remobilization and the development of osteoarthritis, Acta. Orthop. Scand. 47:290, 1976.