Surgical model of osteoarthritis secondary to medial patellar luxation in dogs

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ABSTRACT: This study was performed to make a surgical model of osteoarthritis (OA) in the dog. Experimental medial patellar luxation (MPL) was surgically produced in the left stifle (index) of 24 skeletally mature mixed small breed dogs (age two to six years and weight 2.8 to 9 kg). The animals were randomly allocated in 2 groups; sham group (n = 12), where the right stifle was sham operated and control group (n = 12) with intact right stifle. Physical and radiographic examinations of both stifles were performed at 1.5 months intervals over a one-year experimental period. One dog was euthanatized every three months, and both stifles were explored, gross examination was performed and tissue samples from the articular cartilage, cranial cruciate ligament (CCL) and synovium were collected for histomorphology. The clinical signs of OA were obvious in the experimental dogs by 12 weeks of surgical induction of MPL, which was also evidenced in the histopathology of the joint tissues and electron microscopy of the articular cartilage. The radiographic changes of OA were not obvious until remarkable degenerative changes became abvious six months postoperatively. Surgically induced MPL can be a successful tool for experimental induction of OA in dogs.

Keywords: osteoarthritis; medial patellar luxation; surgical model; dog

Osteoarthritis (OA) is a slow progressive disorder of synovial joints that affects about 20% of the canine population over one year of age. This joint disorder is characterized by a loss of balance between synthesis and degeneration of the articular cartilage constituents leading to subsequent erosion of joint cartilage, remodelling of the underlying bone, osteophyte formation and variable degrees of synovitis (Johnston, 1997). When clinical characteristics of OA (e.g., pain, loss of mobility, and radiographic narrowing of the joint space) are made manifest it means that actual changes in articular cartilage and subchondral bone have started long ago (Marijnissen et al., 2002; Matyas et al., 2004). Because of the difficulty of obtaining normal and OA tissues from the affected joint in humans, a number of animal models (cranial cruciate ligament transection, meniscectomy, creating femoral condylar defect, groove model etc) have been developed (Pond and Nuki, 1973; Lefkoe et al., 1993; Lindhorst et al., 2000).

There are two types of experimentally-induced OA models; chemical and physical. Injection of chemical reagents and biological mediators into the joint of small animals leads rapidly to macroscopic or histopathologic lesions similar to OA. Physically-induced OA models often have rapid and more severe cartilage degeneration than spontaneous models. Surgical induction is mainly performed in larger animals such as dogs, cats and rabbits. Common methods are meniscectomy and transection of the cranial cruciate ligament (CCL), which result in a true instability of the joint leading to OA (Roos et al., 1995). Alterations of the joint load by tibial osteotomy (Panula et al., 1997), by immobilization (Leroux et al., 2001) are other used physical stifle OA models. In the CCL transected model, permanent instability in the stifle joint is followed by degenerative changes in cartilage and synovial tissues in dogs (Brandt et al., 1991). In this model it is not possible to study the changes that take place in the CCL tissues during the pathogenesis

of OA. A femoral condylar defect on the weight-bearing area resulted in progressive osteoarthritic changes in the affected area of the femoral condyle and in the unaffected tibial plateau directly beneath it in the rabbit (Lefkoe et al., 1993). However, OA models where OA is induced by artificial damage to the articular cartilage cannot provide clear information on whether the cartilage erosions are due to the pathogenesis and advancement of the severity of OA or due to the mechanical friction with the artificially made damage during movement of the joint. Considering the above things, in the present study a surgically made medial patellar luxation (MPL) approach was used to induce degenerative changes in the canine stifle. In this model, permanent instability in the stifle joint was induced by a surgically-made MPL, which was followed by degenerative changes in cartilage and changes in synovial tissue that over the course of several months led to canine OA, which resembles human clinical OA. As the CCL and articular cartilage was not manipulated, any changes which occurred postoperatively can be considered to be due to the pathogenesis of OA. In addition, this model can also provide information about whether MPL predisposes to CCL diseases or not.

MATERIAL AND METHODS

Experimental animals

Twenty four skeletally mature healthy mixed small breed dogs of both sexes, age two to six years and weighing 2.8 to 9 kg were used in this experiment. Before engaging in the experiment, physical and radiographic examinations of the animals were performed to ensure the absence of neurologic disease, patellar luxation or other orthopaedic diseases, as well as other surgical procedures performed on either hind limb. The animals were housed in individual cages in the departmental animal shed, Department of Surgery, College of Veterinary Medicine, Chonbuk National University and were fed a standard commercial diet (Precept Adult®, Precept Co., USA) and had water *ad libitum*.

Anaesthesia, surgery and postoperative care

After premedication with atropine sulfate (Atropine Sulfate Inj®, Dai Han Pharmaceutical, Korea) 0.05 mg/kg, *s.c.,* anaesthesia was induced using thiopentone sodium (Thionyl Inj®, Dai Han Pharmaceutical, Korea) 25 mg/kg, *i.v.* and maintained with enflurane and oxygen delivered through a cuffed endotracheal tube.

In all the animals, a left stifle arthrotomy was performed using a standard parapatellar approach. The incision was made through the fascia lata just lateral to the patellar ligament. The joint was thoroughly explored to observe the condition of the cruciate ligaments and menisci. MPL was produced by placing purse string sutures around the parapatellar fibrocartilage and anchoring the patella with the fabellar ligament using a monofilament sterile nylon suture. Medial retinacular reinforcement (imbrication) and lateral release were also performed to interfere the neutral tracking of the patella. In addition, the dogs of the sham group (n = 12) received a sham operation on their right stifles, whereas those in the non-surgical control group (n = 12) remained intact. For sham operation, a scalpel blade was inserted into the right stifle through a lateral parapatellar stab incision to inflict minor trauma to the synovium while the other joint components remained intact.

Post surgically the animals were kept under close observation in a postoperative room and were administered cephalexin (Methilexin Inj®, Union Pharmaceutical, Korea) 25 mg/kg, *i.v.*, every eight hours, for five days and dexamethasone (Dexamethasone Inj®, Daewon Pharmaceutical, Korea) 0.2 mg/kg, *i.v.*, every six hours for three days. The external stitches were removed after healing of the wounds and the animals were moved to the experimental animal shed after 10 days.

Physical and radiographic examinations

Postoperatively physical and radiographic examinations were performed at every one- and half-month interval. Gait, posture, limb function and joint motion were observed. The stifle area was thoroughly examined for the presence of inflammatory signs (swelling, pain, redness, etc). The postoperative radiographs were assessed to ensure a permanent luxation of the patella and to evaluate the development of radiographic signs of OA. The radiographs obtained on different experimental periods were compared and contrasted with the preceding radiographs of the experimental stifles and the contralateral stifles, as well as with those obtained preoperatively.

Gross and histomorphological examination of the joint components

At three-month intervals one dog from each group (control and sham operated right stifles) was euthanatized with the administration of thiopentone sodium (Thionyl Inj®, Dai Han Pharmaceutical, Korea) 75 mg/kg, *i.v.* followed by injection of a massive dose of potassium chloride (KCl-40 Inj®, Dai Han Pharmaceutical, Korea) directly to the heart. After euthanasia, the stifle joints were thoroughly explored with a lateral parapatellar incision to observe the condition of the cruciate ligaments, menisci and articular cartilage. The synovial tissues from the lateral parapetellar site, the CCL and articular cartilage from both the condyle and also from the damaged area (if any) were collected for histomorphology. Immediately after collection, the tissue samples were fixed in 10% buffered formalin. Half of the cartilage specimen was preserved in 2.5% gluteraldehyde for electron microscopy and the other half was demineralized in 8% formic acid for one week. The specimens were dehydrated through graded series of alcohols, embedded in paraffin, cut into 5 µm thick sections, and stained with Hematoxylin and Eosin. Then the slides were examined under light microscope by somebody who was blind to the experiment.

Scanning electron microscopy of the articular cartilage

The articular cartilage specimens from the weight-bearing surface of the medial femoral condyle and also from the damaged area (if any) were collected from both stifles (index and contralateral) from the euthanatized dogs for scanning electron microscopy. Immediately after collection the cartilage specimens were trimmed and primarily fixed in 2.5% gluteraldehyde for three hours at room temperature. Then the specimens were washed in 0.1M sodium cacodylate buffer (pH 7.4) twice and post fixed in 1% osmium tetroxide for two hours at 4 °C. The specimens were washed with the same buffer (sodium cacodylate) twice followed by dehydration through graded alcohol (50%, 70%, 80%, 90% and 100%; 10 minutes in each grade for twice). The specimens were then emerged in 100% ethanol plus 3-methyl butyl (isoamyl) acetate solution at a ratio of 3 : 1, 1 : 1 and 1 : 3 for 10 minutes in each followed by emersion in 3-methyl butyl (isoamyl) acetate solution twice for 10 minutes. The specimens were subsequently dried in liquid carbon dioxide, coated with gold and examined under a scanning electron microscope (JSM 5600 LV, JEOL Ltd., Japan) at different magnifications for abnormalities of the surface of the cartilage by a specialist histomorphologist who was blind to the experiment.

Statistical analysis

The data obtained in the present study were analyzed using ANOVA and Student's *t*-test, and a $P < 0.05$ or less was considered as statistically significant. The data are presented as mean ± standard deviation (SD).

RESULTS

Postoperatively all the animals recovered from the anaesthesia without complications and healing of the surgical wound was quite normal. During the experiment none of the animals were affected with systemic diseases. The activity of the animals was also normal except for a few which were reluctant to walk because of the lameness stemming from the progression of OA.

Physical and radiographic examinations

The physical examination of the animals 12 weeks postoperatively revealed stiffness of gait, lameness, decreased range of motion, and palpable crepitus along with some degrees of joint swelling and pain on the OA-induced limb, whereas the control or sham operated limb was normal. The animals were found reluctant to move and use the OA-induced limb (left). Evaluation of the postoperative radiographs did not show any remarkable changes up to 4.5 months but revealed a permanent medial luxation of the patella. However, radiographs obtained six months postoperatively and onward revealed evidences of OA which included osteophytosis, soft tissue thickening, narrowing of the joint spaces, and subchondral sclerosis in the index stifles, whereas the contralateral stifles (both control and sham operated) were quite normal (Figure 1).

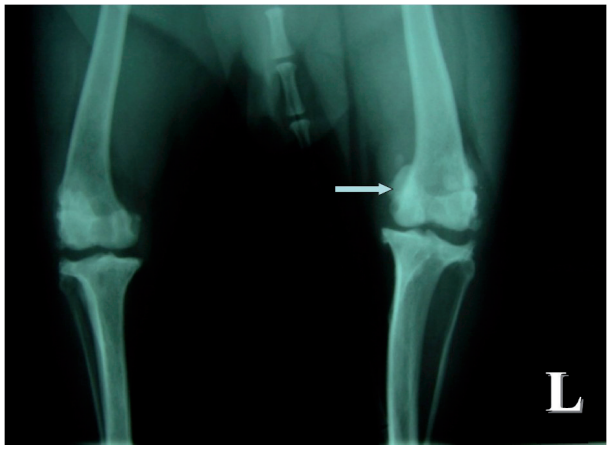

Figure 1. Craniocaudal radiograph of the stifle joints six months after surgical induction of MPL. The right stifle revealed a normal state of the joint, whereas the left stifle (experimental) revealed medially luxated patella (arrow) along with the radiographic changes of osteoarthritis; osteophytosis, soft tissue thickening, narrowing of the joint spaces, and subchondral sclerosis

Gross and histomorphologic changes of the joint tissues

With a lateral parapatellar incision in the euthanatized dogs both the stifles were explored to observe the joint components. In all control and sham operated stifles (right), the articular cartilage was white, shiny, and the surfaces of the femoral and tibial articular cartilage were intact regardless of the time elapsed from the OA induced to the opposite stifles (left). In the index stifles, the medial femoral condyle showed partial thickness cartilage erosions starting from 6 months onward after OA induction, and there were multiple osteophytes around the joint periphery after 12 months (Figure 2). There were no signs of gross cartilage surface fibrillation on the tibial surfaces in the index joints. The articular cartilage of the index stifles appeared more opaque and thicker than the cartilage of control and sham operated stifles, which appeared thinner and translucent.

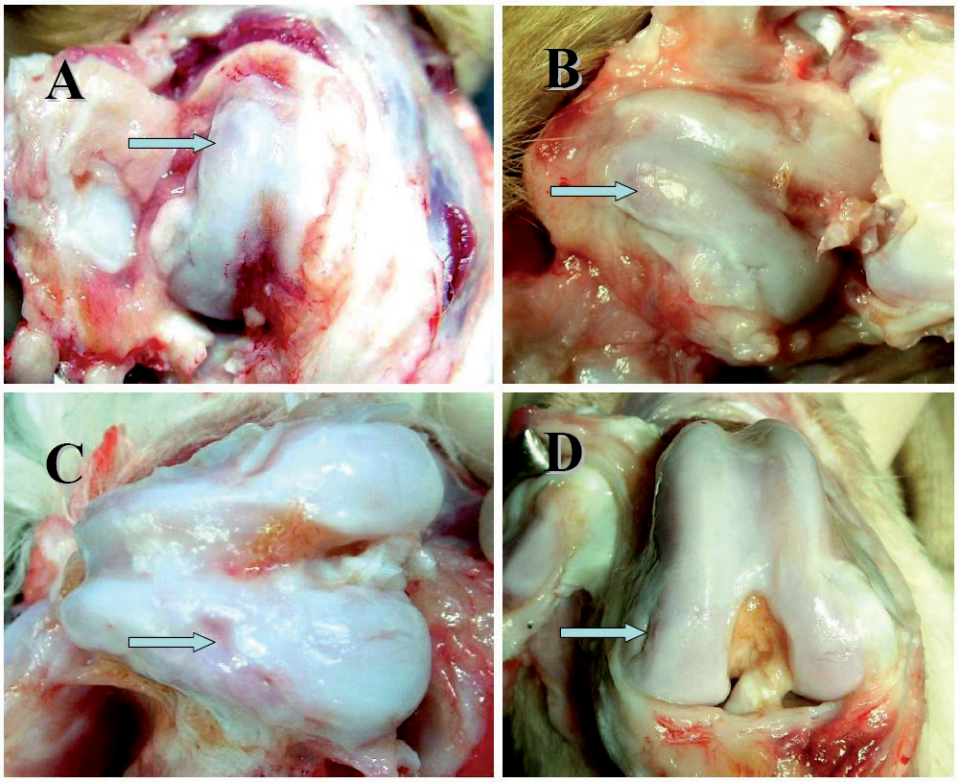

Figure 2. The medial condyle shows cartilage erosion 6 months after induction of OA (**A**). The medial condyle shows full thickness cartilage erosion and opacity of the articular cartilage 9 months after induction of OA (**B**). Severe articular cartilage erosions and formation of multiple osteophytes 12 months after induction of OA (**C**). Gross appearance of the CCL six months postoperatively revealed fibrous thickening of the ligament (**D**)

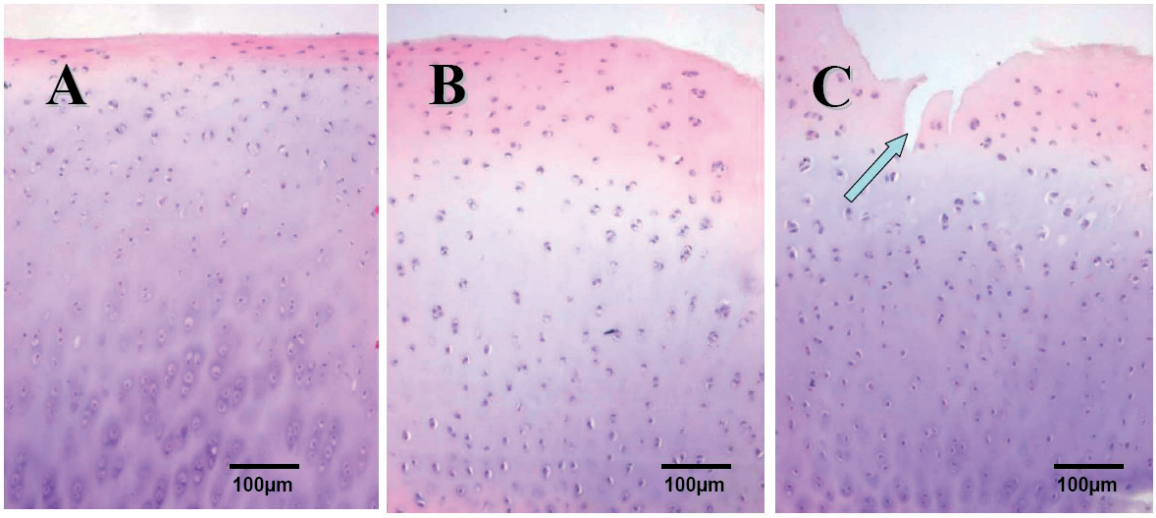

Figure 3. Photomicrograph of the articular cartilage collected from the weight-bearing area of the medial femoral condyle; control (**A**), six months (**B**), and nine months after induction of OA (arrow indicated fissuring of the articular cartilage) (**C**); Hematoxylin and Eosin stain

The longer the period from the induction of OA, the more the degrees of cartilage erosion were observed. The gross appearance of the CCL in the control and sham operated joints was normal, whereas that in the index stifles revealed fibrous thickening and defibrillation of the ligament fibres which progressed with time (Figure 2). Partial rupture of the CCL was found in two OA-induced stifles.

The histological appearance of the cartilage from the control and sham operated joints of the dog euthanatized one year after induction of OA was normal with an even surface, whereas the cartilage of the index stifle revealed focal disruptions of the cartilage surface appear with fissuring, resulting in fibrillation of the joint surface (Figure 3). The synovium (Figure 4) and CCL (Figure 5) of the control and sham operated joint revealed mild infiltration of mononuclear cells, whereas those of the OA-induced stifle revealed a markedly increased infiltration of the mononuclear reactive cells. A marked increase in cellularity was also noticed in the synovium of the OA-induced stifles.

Changes in the scanning electron microscopic view of the articular cartilage surface

The development of articular surface changes of the cartilage collected from the weight-bearing area of the medial femoral condyle and damaged area (if any) was examined with SEM. During the development of OA the normal undulations and fine regular fibre network disappeared, the number of

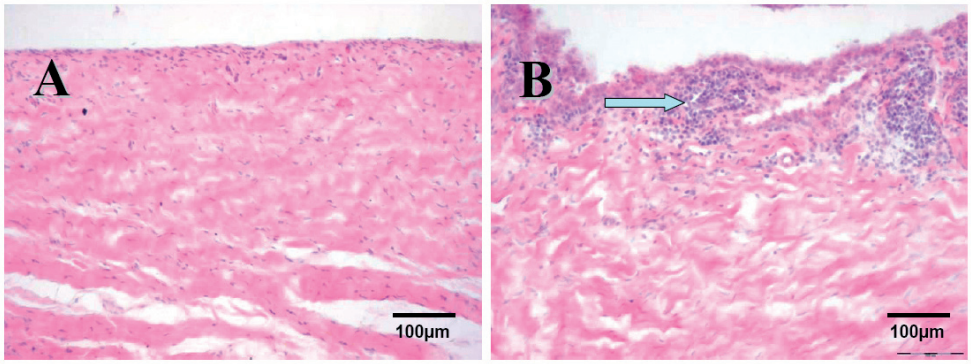

Figure 4. Photomicrograph of the synovium revealed marked proliferation of the lining cells along with infiltration of mononuclear cells (arrow) one year after induction of OA (**B**), while that of the sham operated stifle revealed mild mononuclear cell infiltration (**A**); Hematoxylin and Eosin stain

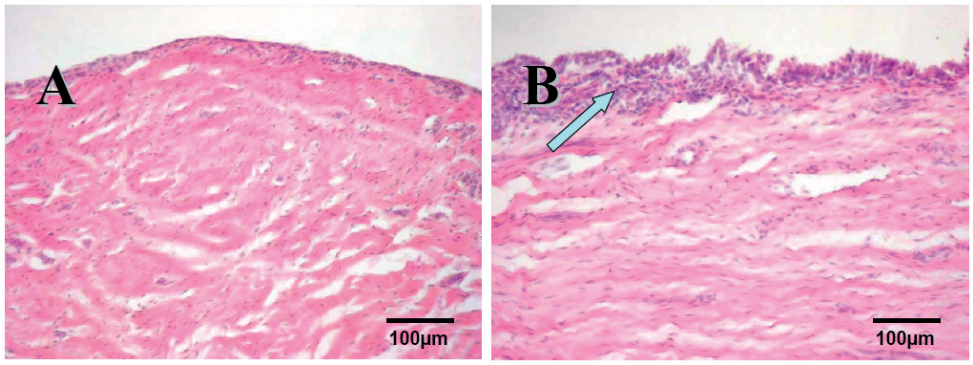

Figure 5. Photomicrograph of the CCL, revealed marked infiltration of mononuclear cells (arrow) one year after induction of OA (**B**), while that of the sham operated stifle revealed mild mononuclear cell infiltration (**A**); Hematoxylin and Eosin stain

fibres and the variation in the thickness of the fibre bundles increased, and scaly irregularities or cracks appeared on the surface of the articular cartilage (Figure 6). The fissure penetrated more deeply into the cartilage at the advanced stage of OA.

DISCUSSION

Animal models are widespread in orthopaedic research to investigate effects of traumatic injuries on articular cartilage *in vivo*, to study the pathological variation in disease progression or to evaluate the potential of disease-modifying drugs (Knecht et al., 2006). The main advantage of animal models is, besides the well-defined time course, the easier access to the joint and its tissues, which enables the quantification of the disease progression. Limitations are ethical issues, high costs, slow time course with large animals and the physiological and anatomical differences between animals and humans (Griffith and Schrier, 2003).

The present study demonstrated that surgically-made MPLs, result in clinical signs and histological changes similar to those seen in the canine CCL transected and groove model of OA (Pond and Nuki, 1973; Marijnissen et al., 2002; Mastbergen et al., 2006). The difference is that in the MPL model and CCL transected model, the process of OA remains affected by continuous joint instability whereas in the groove model, it is the perpetuation of the initial cartilage damage which is intrinsic to the process of OA (Marijnissen et al., 2002). In this study, the characteristic clinical signs of OA; joint pain, limitation of movement, effusion and variable degrees of local inflammation were noticed by three months after the induction of OA. These finding are in agreement with the previous reports on the animal models of OA (Pond and Nuki, 1973; Lefkoe et al., 1993; Marijnissen et al., 2002). The early stages

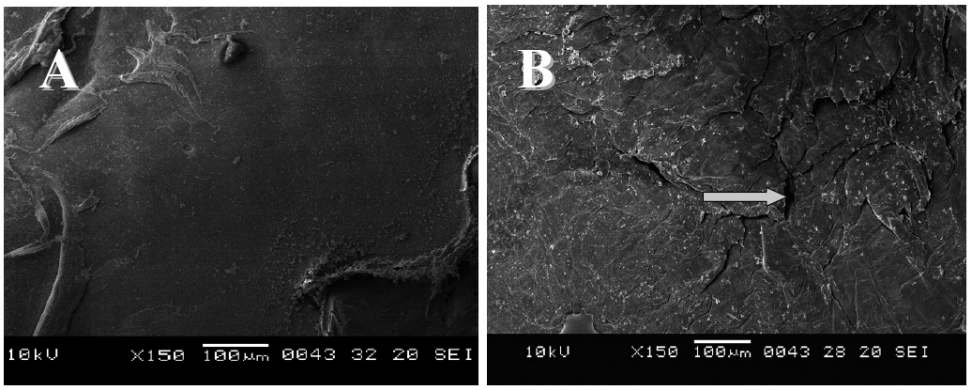

Figure 6. Scanning electron microscopic view (×150) of the articular cartilage surface which was collected from the weight-bearing area of the medial femoral condyle of the contralateral sham operated stifle one year postoperatively revealed an even surface (**A**), whereas that of the medial femoral condyle of the index stifle revealed scaling and loss of smoothness (arrow) of the cartilage surface (**B**)

of OA are difficult to diagnose with radiography. Radiographic changes include joint effusion, osteophytosis, soft tissue thickening, narrowing of the joint space, and subchondral sclerosis which occur only at the later stages of OA (Matyas et al., 2004). In this study, remarkable radiographic changes were also not observed in the early stages of the disease. However, the radiographic changes were more obvious with the progression of OA after the surgical induction of MPL. These findings are in agreement with the report of Matyas et al. (2004).

It has also been suggested that disruption or loosening of the collagen network in the superficial zone of articular cartilage is closely involved in the initiation of OA (Guilak et al., 1994). Decreases in the superficial proteoglycan concentration and superficial collagen network disruption coincide with softening of the articular cartilage (Bentley, 1985). Later, focal disruptions of the cartilage surface appear with fissuring, resulting in fibrillation of the joint surface. Fissures penetrate more deeply into cartilage and eventually down to the subchondral bone. This leads to further roughening of the cartilage surface until complete abrasion of the joint surface develops (Maroudas and Venn, 1977). The macroscopic, microscopic and radiographic changes occurring in typical OA were fully evident in the MPL model of OA. However, the mechanism of how MPL induces OA is not yet fully understood. It has been thought that the MPL causes permanent instability in the stifle joint impairing the normal tracking of the patella in the femoral trochlea and loss of stabilization forces of the quadriceps muscle group against cranial drawer motion (Hayes et al., 1994). The loss of the stabilization force of the quadriceps muscle group is hypothesized to cause angular and rotational deformities in the distal part of the femur and tibia which place abnormal stresses on the articular cartilage and CCL, and with the progression of time this may lead to the pathogenesis of OA in dogs with MPL. Saito et al. (1991) and Ryu et al. (1997) reported the progression of degenerative changes of articular cartilage in patellar luxation with time in a rabbit model of patellar luxation. Roy et al. (1992) also reported OA in dogs with patellar luxation in a retrospective study. The gross, microscopic and radiographic changes which occurred in this study are in agreement with the previous reports of OA in a patellar luxation model (Saito et al., 1991; Roy et al., 1992; Ryu et al., 1997) and also with those of the conventional animal models of OA (Pond and Nuki, 1973; Lefkoe et al., 1993; Lindhorst et al., 2000; Marijnissen et al., 2002). CCL rupture secondary to MPL has been thought to be due to malalignment of the extensor mechanism of the stifle and internal rotation of the proximal tibia. CCL tears in association with patellar luxation have been proposed to be caused at least in part by loss of the normal stabilizing forces of the quadriceps muscle group against cranial drawer motion. Angular and rotational deformities in the distal part of the femur and proximal part of the tibia are also hypothesized to place abnormal stress on the collateral ligaments and CCL; these stresses combined with the normal aging (degenerative) process in these structures, could account for the findings of CCL tears in dogs with patellar luxation (Alam et al., 2007).

The contralateral joint of each dog was used as a control (sham operated or non-surgical). Others have shown that sham operations gives results similar to those for unaffected control knees in the CCL transected model (Pelletier et al., 1985). As described by Myers et al. (1990), the severity of the pathologic changes varies in many laboratories, which could be related to differences in the animal's age, weight or breed and the postoperative exercise regimen, or the length of time from surgery to euthanasia of the animal. In this study, mixed bred small dogs were used and the variation in the age and weight was not marked. The advantages of using small dogs are the ease of handling physical and radiographic examinations, and the collection of SF and blood samples, while they also require only little food and space for housing.

Animal models are essential in research on OA to better understand the pathophysiology of OA especially in its early phases and to study the therapeutic effects of antiarthritic drugs. The physical and radiographic examinations, gross and histomorphological findings of this study suggest that surgically-made MPL can induce OA in the canine stifle which is similar to the pathophysiology of OA observed in other conventionally used animal models of OA. The MPL model can be a successful tool for inducing OA and for studying the early degenerative changes in the pathogenesis of the disease.

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