Study of the Effect of Excessive Tibial Plateau Angle on Degenerative Changes of Canine Cranial Cruciate Ligament

Summary of the Doctoral Thesis

Tom Ichinohe

Graduate School of Veterinary Medicine and Life Science

Nippon Veterinary and Life Science University

Canine cranial cruciate ligament rupture (CrCLR) is a very common orthopaedic disease of the canine stifle. The cranial cruciate ligament (CrCL) prevents cranial tibial displacement relative to the femur, excessive internal tibial rotation, and stifle hyperextension. Cranial tibial thrust (CrTT), which is a force generated during hind limb weight bearing, and an abnormally increased stifle internal rotation may both result from CrCLR. Dogs affected with CrCLR may subsequently develop progressive stifle osteoarthritis and secondary meniscal damage. Although anterior cruciate ligament (ACL) rupture can occur acutely in humans due to trauma, most canine CrCLRs occur secondary to chronic degenerative changes in the CrCL. The degenerative changes in the CrCL are characterized by the degeneration of the extracellular matrix (ECM) in the CrCL, eventually leading to ligament rupture secondary to non-contact injury. Multiple histologic changes, including decreased cell density, disorganization of collagen fibres, and phenotypic changes in ligamentocytes, have been reported in the degenerated CrCL. One key histological characteristic is the alteration in ECM, particularly chondroid metaplasia. Excessive tibial plateau angle (eTPA; TPA≧35°), which converts more weight loading to the cranial tibial thrust than the normal tibial plateau angle (TPA) and increases the tensile force in the CrCL, is recognized as one of the risk factors of CrCLR. To date, there are no known studies investigating the relationship between the eTPA and degenerative changes in the CrCL. We hypothesized that degenerative changes in CrCLs, such as chondroid metaplasia, were increased in the stifles with eTPAs.

The objective of this study is to investigate the effect of eTPA on the degenerative changes. In the chapter 2, in order to evaluate the utility of implants for the TPA increasing procedure to generate a model animal for eTPA, we measured the changes of the TPA in cases in which the proximal tibial cylindrical osteotomy was performed with various types of implants. In the chapter 3, we aimed to measure the tensile force in the CrCL, medial and lateral collateral ligament (MCL and LCL) in normal canine stifles and artificial stifle models of eTPA and evaluate the effect of the TPA increasing procedure on the tensile force of these ligaments. In the chapter 4, we aimed to describe the development of chondroid metaplasia, the changes in the expression of ECM components, and the expression of the Sry-type HMG box 9 (SOX9),

which is the key factor for the cartilage differentiation and the expression of the cartilage matrix, in CrCLs affected by CrCLR in dogs. In the chapter 5, we aimed to generate an animal model of eTPA and evaluate degenerative changes of the CrCL and the caudal cruciate ligament (CaCL).

2. The effect of plate types on tibial plateau angle and mechanical medial proximal tibial angle after tibial plateau leveling osteotomy.

Various types of specialized plates are available for the corrective osteotomy that change the proximal tibial shapes such as tibial plateau leveling osteotomy (TPLO) which changes TPA by the osteotomy in the proximal tibiae. In order to evaluate the utility of these implants for the TPA increasing procedure, we measured TPA and mechanical medial proximal tibial angle (mMPTA) in cases in which TPLO was performed with a Slocum plate (SP), locking compression TPLO plate (LCP), and dynamic compression plate (DCP). The TPA and mMPTA were then compared among different types of plates and after each surgical procedure. The TPA at 1, 2, and 3 months was significantly higher than that immediately after surgery in the SP group. There were no changes in the postoperative TPA over time in the LCP group. The TPA at 2 and 3 months was significantly higher than that immediately after surgery in the DCP group. There were neither changes in the postoperative mMPTA over time in any group nor any significant difference in the mMPTA among the three groups. Compared with SPs or DCPs, LCPs are very useful to maintain the alignment of the proximal tibial fragment, and DCPs are inferior to LCPs specialized for proximal tibial corrective osteotomy in terms of maintenance of the alignment of the proximal tibial fragment. From this result, we decided to apply LCPs to the TPA increasing procedure.

3. The effect of the tibial plateau angle increasing procedure on the tensile force of the cranial cruciate ligament in the canine femorotibial joint.

We aimed to measure the tensile force in the CrCL, medial and lateral collateral ligament (MCL and

LCL) in normal canine stifles and artificial stifle models of eTPA and evaluate the effect of the TPA increasing procedure on the tensile force of these ligaments. Cadaveric stifles (n = 16) were harvested from normal beagles and allocated into (1) the unchanged tibial plateau angle group (normal group; TPA = 31.2°) or (2) the excessive TPA group (eTPA group; TPA = 41.1°). The eTPA group underwent curvilinear osteotomy at the proximal tibia to increase the TPA. A robotic system applied a 30 N or 60 N compressive force to the specimens. The craniomedial band (CrMB), caudolateral band (CaLB), MCL, and LCL were sequentially transected and the protocol was repeated. Orthogonal force components were measured and the ligament forces calculated after repeated force measurements as ligament contributions were subtracted by transection. As the compressive force increased, the tensile forces in the CrMB and CaLB also increased, but they remained unchanged in the MCL and LCL. The CrMB tensile force was larger in the eTPA group than in the normal group, and the MCL and LCL tensile force were not larger in the eTPA group than in the normal group. An eTPA may increase the stress on the CrCL, but not the MCL and LCL. The TPA increasing procedure used in this study increases the tensile force in the CrCL without showing a major impact on the MCL and LCL.

4. Degenerative changes of the cranial cruciate ligament harvested from dogs with cranial cruciate ligament rupture

We aimed to describe the development of chondroid metaplasia and the changes in the expression of ECM components in CrCLs affected by CrCLR in dogs. CrCLs from 26 stifle joints with CrCLR (CrCLR group) and normal CrCLs from 12 young beagles (control group) were examined histologically and immunohistochemically for expression of type I (COLI), type II (COLII), type III (COLIII) collagen, and SOX9. Cell density and morphology of CrCLs were quantified using HE staining. In CrCLs, the percentage of round cells was higher in the CrCLR group than in the control group. COLI-positive areas were seen extensively in the connecting fibers, but weakly represented

in the cytoplasm of normal CrCLs. In the CrCLR group, there were fewer COLI-positive areas, but COLI-positive cells increased. The percentages of COLII-, COLIII- and SOX9-positive cells were higher in the CrCLR group than in the control group. Deposition of COLI, the main ECM component of ligaments, decreased with increased COLIII expression in degenerated CrCL tissue, which shows that the deposition of the ECM is changed in degenerative CrCL disease. There was no significant correlation between the period from the onset of clinical sign and the expression of COLI, COLI, COLI, and SOX9. On the contrary, expression of SOX9 increased, which may contribute to the synthesis of cartilage matrix. The expression of COLII and SOX9 in ligamentocytes showed that these cells tend to differ to chondrocytes. It is reported that the chondroid metaplasia is s a physiological, not pathological, response, and there is no correlation with the period from the onset of the clinical sign. Therefore it is suggested that these changes occur before the ligaments rupture, although the possibility cannot be denied that ligament rupture have effects on these changes.

5. Degenerative changes of the cranial cruciate ligament harvested from dogs with cranial cruciate ligament rupture

We aimed to generate an animal model of eTPA and evaluate degenerative changes of the CrCL and the caudal cruciate ligament (CaCL). Seven mature female Beagles were included. Cylindrical osteotomy was performed bilaterally in the proximal tibia. The TPA was increased to approximately 40° in the left tibia (eTPA group) and remained unchanged in the right tibia (control group). The dogs were subjected to exercise stress beginning 3 months postoperatively and were euthanized 12 months postoperatively, and the CrCLs and CaCLs were collected. All specimens were stained with haematoxylin and eosin (HE) to assess the cell morphology and subjected to immunostaining to evaluate COLI, COLII, and COLIII, and the SOX9 expression. Macroscopic CrCL injury was absent in 6 dogs, but was present in one in the eTPA group, which was excluded from the analysis. The cell density decreased and the percentage of round cells increased in the eTPA group. In the

eTPA group, there were fewer COLI-positive areas, but COLI-positive cells increased. The percentages of COLII-, COLIII- and SOX9-positive cells were higher in the eTPA group than in the control group. There was no significant difference in the CaCLs between the eTPA group and the control group.

The degenerative changes of the CrCL with eTPA in this study were similar to the degenerative changes in the CrCL from the cases affected by CrCLR. Moreover these changes were not observed in the CaCL with eTPA. It is reported that tensile force in the fibrocytes increase lead to the expression of the SOX9 increase. Therefore the eTPA may increase the tensile force in the CrCL and may increase the expression of the SOX9 and may increase the expression of the cartilage matrix such as COLII. It is also proposed that cartilage-like tissue is more vulnerable to disruption under normal tensile forces, and any fibrocartilaginous transformation may predispose to injury of the CrCL. In the eTPA group, there were fewer COLI-positive areas, but COLI-positive cells increased similar to the degenerative CrCL from the cases affected by CrCLR. It is reported that the turnover of COL I, the principal tensile-resistant fiber increases when the tensile force in the ligamentocyte in the CrCL increases, and that immature collagen crosslinks are increased in ruptured CCLs, which may contribute to a decrease in tensile strength. Therefore the eTPA may increase the tensile force in the CrCL and may increase the turnover of the COL I and may contribute to a decrease in tensile strength of CrCL. In the eTPA group, COLIII-positive cells increased similar to the degenerative CrCL from the cases affected by CrCLR. Increased expression of COLIII is the first step during injury healing, which is then finally replaced with COLI. Therefore eTPA may increase the tensile force in the CrCL and may increase the turnover of the COL III because of micro injuries increase in the CrCL and may contribute to a decrease in tensile strength of CrCL.Taken together, these observations suggested that eTPA may promote the degenerative changes in the CrCL and may be one risk factor of CrCLR.