Role of Ligamentous Restraints During Anterior-Posterior Drawer Tests of the Murine Knee

Snehal Shetye, PhD[1]
John Bova, BS[1]
Andrew Kuntz, MD[1]
Miltiadis Zgonis, MD[1]
David Butler, PhD[2]
Nathaniel Dyment, PhD[1]

[1]McKay Orthopaedic Research Laboratory
University of Pennsylvania

[2]Biomedical Engineering Program
University of Cincinnati

Introduction

Murine models of altered knee loading are frequently employed to study the pathogenesis of osteoarthritis and more recently, to investigate tendon-bone attachments within the ensuing bone tunnels.[1,2] Human cadaveric and large animal model demonstrated that the anterior cruciate (ACL) and posterior cruciate (PCL) ligaments are the primary restraints to anterior or posterior tibial translation, respectively. Even though murine knee destabilization models are common, the specific contributions of the cruciate ligaments have not been quantified. Further, the role of other murine knee ligaments that provide secondary restraints, such as the medial and lateral collateral ligaments (MCL, LCL) under anterior/posterior loading remains unknown. Therefore, the objective of the study was to investigate the role of ligamentous restraints in the murine knee during anterior-posterior loading. We hypothesized that the ACL and PCL will be the primary restraints to anterior and posterior drawer, respectively. Based on murine knee ligament anatomy, we also hypothesized that the MCL will be the secondary restraint in anterior drawer and the LCL will be the secondary restraint in posterior drawer.

Methods

Experimental Design

All animals and procedures were approved by UPenn's IACUC. CD1 mice (4 male, 3 female, n = 7) 16 weeks of age were assessed for anterior-posterior drawer stability in a custom fixture recently described by our group.[2] Briefly, following sacrifice, 7 hindlimbs (6 right, 1 left) were isolated, all extraneous soft tissue removed, and all capsule ligaments, including the cruciates and collaterals, along with the menisci left intact. The patellar tendon was removed based on its previously described role in anterior posterior loading.[3] The distal half of each tibia was potted in an acrylic tube using PMMA. This construct was then loaded onto a material testing machine. The potted tibial end was fixed in a custom fixture that allowed for adjustment of tibial plateau angle. The distal end of the femur was lowered into another acrylic tube affixed to a custom fixture that could control knee flexion by rotating the femur around the joint center of rotation. The knee was set up at 90 degrees of flexion for all tests. The knee joint was tested for anterior and posterior stability by cyclic loading under displacement control between ± 0.3mm for 5 cycles and the 5th cycle was used to quantify stability for all cases

Selective Cutting Procedure

The intact knee joint loads were evaluated at ± 0.3mm (+ve = anterior drawer, -ve = posterior drawer) to establish the baseline conditions. Following this, a 27G needle was used to carefully cut the ACL with an anterior approach. This procedure was performed on the testing machine itself to not lose the initial knee joint zero reference position. The stability test was repeated at ±0.3mm to quantify the contribution of the ACL. Next, the PCL was carefully transected, and the stability test repeated to quantify PCL contribution. After transection of the ACL and PCL, the knee joint was substantially unstable and barely registered any loads at ± 0.3mm. Thus, to quantify MCL and LCL contributions, the drawer tests were modified to reach ±1.0mm, as described previously in cadavers.[3] This allowed for quantifying MCL and LCL contributions as secondary restraints. The transection of each ligamentous structure was not randomized since it has been shown previously to not have an effect if tests are conducted under displacement control to a specified peak displacement.[3]

Results

Primary Restraints

The peak anterior restraining force for the intact knee was 1.24 ± 0.17N at 0.3mm of displacement. The peak posterior restraining force for the intact knee was 0.82±0.1N at −0.3mm of displacement. Transection of the ACL dropped the peak anterior restraining force at 0.3mm to 0.06 ± 0.04N indicating that ACL contributed to 95.01 ± 3.30% of the restraining force. Interestingly, ACL transection also reduced the peak posterior load by 14.0 ± 9.83%. Transection of the PCL dropped the posterior restraining force at −0.3mm to −-0.1 ± 0.07N, which translated to a contribution of 89.55 ± 6.96% of the peak restraining force in the posterior direction at -0.3mm by the PCL. Primary restraint contributions can be seen from a representative sample in Figure 1.

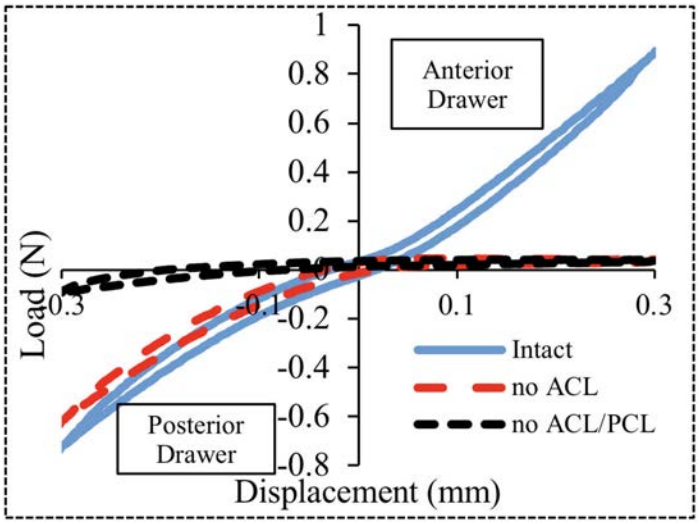

Figure 1. Representative sample plots that show the effect of transecting the ACL and PCL on anterior and posterior drawer loads.

Table 1: **ACL and PCL force contributions at 0.3mm drawer. Values are reported as Mean (S.D.).**

Anterior Drawer (n=7)			Posterior Drawer (n=5)			
Intact Force	Anterior Cruciate Force	ACL Contribution	Intact Force	Posterior Cruciate Force	PCL Contribution	ACL Contribution
(N)	*(N)*	*(%)*	*(N)*	*(N)*	*(%)*	*(%)*
1.24(0.17)	1.18(0.18)	95.01(3.3)	−0.82(0.1)	−0.58(0.09)	89.55(6.96)	14.0(9.83)

Secondary Restraints

After ACL and PCL transections, the peak restraining forces at ± 1.0mm of displacements were found to be 0.32 ± 0.19N and −0.52 ± 0.31 in the anterior and posterior directions, respectively. Transection of the MCL only influenced the anterior peak force whereby the force dropped by 86.13 ± 8.3%. Transection of the LCL only influenced the posterior peak force whereby the force dropped by 85.12 ± 11.89%.

Discussion

To better understand molecular and genetic mechanisms that regulate osteoarthritis pathogenesis in murine models, it is crucial to understand the mechanical stability of the knee joint before and after destabilization and how the joint adapts with time post-injury. The data presented here provide a baseline for studies creating these OA models by transecting supporting ligamentous structure involved in knee stability. We found that, similar to the human knee, the ACL and PCL are the primary structures providing anterior and posterior stability, respectively, in the murine knee. Interestingly, we did see an approximately 14% contribution by the ACL to posterior stability, which has not been observed in humans. Further, at a knee flexion angle of 90 degrees, and in absence of the ACL and PCL, the MCL provides most of the stability in the anterior direction and the LCL provides most of the stability in the posterior direction. While great care was taken to not disrupt other structures such as the menisci during transection of these ligaments, it is possible due to the small size of the murine knee joint. However, future studies will use contrast MicroCT to verify damage to each structure. Our data suggest that the menisci did not play a major role in anterior-posterior knee stability since the peak load were close to 0N after transection of these ligaments. This agrees with a previous study that followed a displacement control protocol as presented here.[3] However, other studies that employed a load-control protocol have shown that the menisci play a role in knee stability at lower flexion angles.[4,5] Furthermore, our study did not apply a compressive load to the knee, which might be necessary for the menisci to be loaded. Alternatively, a case could also be made for the menisci to be more important in varus-valgus knee stability, which was not investigated here. Further studies will investigate the influence of knee flexion angle, compression joint load, and the menisci to anterior-posterior stability of the murine knee.

Significance/Clinical Relevance

This methodology can be applied to murine knee destabilization PTOA models over the time course of OA progression, correlating with biological changes to the joint. In addition, methods to restabilize the knee[2] to attenuate OA progression can be verified.

Acknowledgements

This study was supported by the Penn Center for Musculoskeletal Disorders P30 grant (AR069619), the NIH R00 award (AR067283), and the Thomas B. McCabe and Jeannette E. Laws McCabe Fund at UPenn.

References

1. Blaker CL, *et al.* *J Orthop Res,* 2017 (35)424–439
2. Kamalitdinov TB, *et al.* *J Orthop Res,* 2019
3. Butler DL, *et al.* *JBJS,* 1980 (62)259-270
4. Markolf KL, *et al.* *JBJS* 1976 (67)136-146
5. Shoemaker SC, *et al.* *JBJS* 1986 (68)71-79