Comp Clin Pathol (2010) 19:185–188
DOI 10.1007/s00580-009-0843-6

ORIGINAL ARTICLE

Ostrich tendon (new xenogenic) transplantation in rabbit model

A. S. Bigham · A. K. Zamani Moghaddam · H. Nourani · M. Cheraghchi Bashi · A. Raeisi

Received: 4 February 2009 / Accepted: 11 March 2009 / Published online: 28 March 2009

Abstract Since tendons are subjected to repeated motion and degeneration over time, they are prone to both acute and chronic injuries. In addition, the blood supply to the tendon is generally poor; as a result, healing often progresses slowly. Autogenic, allogenic and xenogenic tendon transplantations have been performed in reconstructive tendon surgery. There is no report on ostrich (xenograft) tendon transplantation to other species. The aim of this study was to evaluate ostrich flexor tendon transplantation in a rabbit model. Seven male New Zealand white rabbits 1 year old and weighing 4.0±0.5 kg were used in this study. Approximately 3 cm of the superficial flexor tendon was resected, and created defects were filled in all rabbits with 3-cm harvested ostrich chick tendon and sutured with 2/0 polypropylene in a single Modified Kessler suture pattern. The main histopathological and gross evaluation showed graft necrosis and sequestration. Our results showed that ostrich tendon displays severe antigenicity and elicits a vigorous inflammatory reaction and is, therefore, not recommended for use as a xenogenic tendon graft for the replacement of tendons or ligaments.

A. S. Bigham (✉) · M. Cheraghchi Bashi · A. Raeisi
Department of Veterinary Surgery and Radiology,
Faculty of Veterinary Medicine, Shahrekord University,
Shahrekord, Iran
e-mail: dr.bigham@gmail.com

A. K. Zamani Moghaddam
Department of Poultry Science,
Faculty of Veterinary Medicine, Shahrekord University,
Shahrekord, Iran

H. Nourani
Department of Veterinary Pathobiology,
Faculty of Veterinary Medicine, Shahrekord University,
Shahrekord, Iran

Keywords Xenogenic · Tendon transplantation · Ostrich tendon · Rabbit model

Introduction

Tendons are soft connective tissues consisting of parallel collagen fibres embedded within an extra-cellular matrix (Lin et al. 2004). Tendons connect muscle to bone and transmit tensile force generated by muscles to move and stabilise joints. They must be capable of resisting high tensile forces with limited elongation (Best et al. 1989; Buckwalter and Hunziker 1996). However, as tendons are subjected to repeated motion and degeneration over time, they are prone to both acute and chronic injuries (Lin et al. 2004). Barfred (1973) suggested that Achilles tendon ruptures can occur in a normal tendon if an excessive load is applied (Barfred 1973). Blood supply to the tendons is reported to be poor; therefore, healing often progresses slowly (Clancy 1983; Harner et al. 1995; Kuo et al. 2003). The healing process in tendons results in the formation of a fibrotic scar. The structural, organisational and mechanical properties of this healed tissue are inferior to normal tendon (Frank et al. 1999). Although these properties improve over time, they do not return to normal levels, even after long periods (Frank 1996; Frank et al. 1999). Rogers et al. (1995) proposed that xenografts are highly attractive as they carry small risk of infectious disease, do not compromise the patient's remaining tissues and may have the same structure as the component being replaced (Rogers et al. 1995). Autogenic, allogenic and xenogenic tendon transplantations have been done previously (McMaster 1985; Rodeo 1993; Strickland et al. 2003). Also, artificial tendon has been used in man (Dong and Sheng 1988). Autogenic and allogenic transplantation of tendons have

been performed with varying degrees of success (Taniguchi and Tamaki 2000). Hamstring tendon graft has been used for anterior cruciate ligament reconstruction (Gordia and Grana 2001). Transplantation of bovine foetal tendon in a rabbit model has been performed successfully (Dehghani et al. 2005). Repair of tendon gap by bovine foetal tendon transplant in the horse has been also done before (Dehghani and Varzandian 2007). Karakurum et al. (2003) previously showed that the ostrich tendon has excellent strength during biomechanical evaluations (Karakurum et al. 2003). They showed that flexor tendons of the ostrich are an excellent source of tendons of good quality and have adequate length. There is no report on ostrich (xenograft) tendon transplantation in other species. Therefore, the purpose of this study was to evaluate ostrich flexor tendon transplantation in a rabbit model.

Materials and methods

Preparation of ostrich tendon

One-week-old ostrich chick cadavers were referred to the Department of Poultry Science for diagnosis of nutritional deficiency. Digital flexor tendons were retrieved aseptically through a paratendinous incision of approximately 5 cm, transferred to a sterile container, cut and shaped immediately and preserved in sterile saline solution at −20 . Prior to transplantation, the preserved tendons were thawed at room temperature for about 1 h (Scottish National Blood Transfusion Service (SNBTS) Tissue Service 2005).

Animals

Seven male New Zealand white rabbits, 1 year old and weighing 4.0±0.5 kg were used in this study. The research protocol for this experiment was approved by the University Research Committee.

Surgical technique

Animals were anaesthetised with ketamine (40 mg/kg, IM) and xylazine (5 mg/kg, IM). The left or right hind leg (randomly selected) was shaved and prepared aseptically with povidon iodine and the limb draped with sterile drapes. The skin was incised on the lateral part of distal third of the tibia over the tendons; the fascia and tendon sheath were incised. About 3 cm of the superficial flexor tendon was resected, and the created defect was filled with 3 cm harvested ostrich chick tendon and sutured with 2/0 polypropylene in a single Modified Kessler suture pattern (Fig. 1). The tendon sheath was sutured over the transplanted tendon completely in all groups.

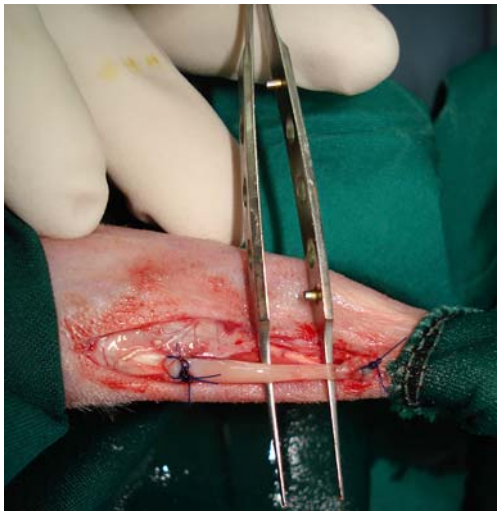

Fig. 1 Ostrich tendon transplantation in a rabbit

Post-operative evaluation

The operated leg was bandaged after the operation and post-recovery; the rabbits were individually housed in a restricted area to limit their movement. Clinical parameters including appetite, activity, infection, bleeding and wound dehiscence were evaluated daily.

Histopathological evaluation

Fifteen weeks after the operation, the rabbits were euthanised pharmacologically for histopathological evaluation. For all rabbits, the graft-tendon unit was resected, fixed in 10% formalin and processed to wax. Two 5-μm sections were cut from the centre of each specimen and were stained with haematoxylin and eosin. The sections were individually evaluated by a pathologist blinded to the treatment.

Results

Clinical evaluation

All rabbits showed normal activity and appetite, and there was no evidence of clinical complications such as local infection or wound dehiscence. In all rabbits, the skin was freely movable across the implant site.

Gross evaluation

The transplanted tendon was difficult to determine as the transplant area lacked any continuity or solid integration and had a yellowish colour in appearance, with evidence of graft contraction and degeneration (Fig. 2).

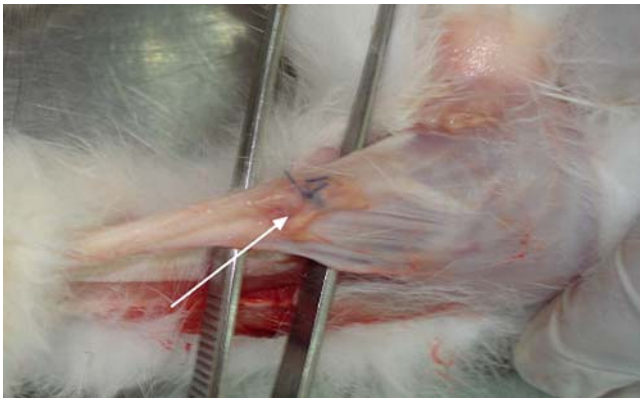

Fig. 2 Note yellowish degenerated xenogenic (ostrich tendon) tendon graft (*white arrow*)

Histopathological finding

The main histopathological findings observed in all animals with the xenograft consisted of graft necrosis and sequestration (Fig. 3). A large number of macrophages or epithelioid cells, eosinophils and foreign body-type giant cells were seen around the necrotic debris (Fig. 4). These structures were surrounded with collagenous connective tissue infiltrated by lymphocytes and plasma cells.

Discussion

In this study, a superficial digital flexor tendon defect model was created to evaluate ostrich tendon transplantation as a new xenograft in a rabbit model. The Achilles tendon or common calcaneal tendon has its origin in five muscles that give rise to three tendinous components: the gastrocnemius, superficial digital flexor, biceps femoris, gracilis and semitendinosus (Dyce et al. 1996). Complete rupture of the Achilles tendon is rare in domesticated animals. Rupture of the gastrocnemius tendon and the superficial and deep flexor tendons are more common in small animals and large animal practice (Bertone 1995).

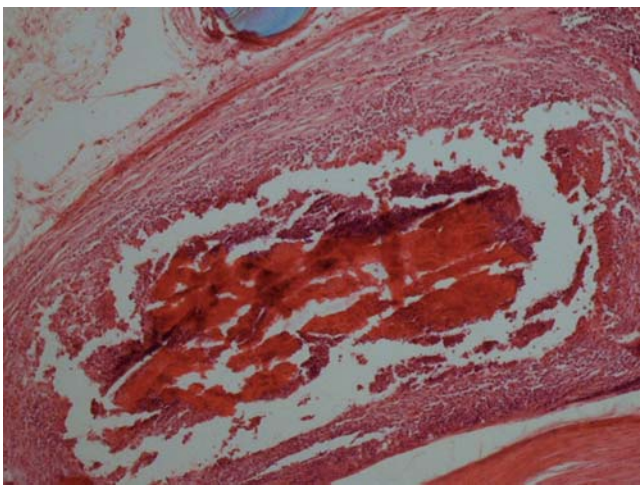

Fig. 3 Xenograft necrosis and sequestration (haematoxylin and eosin, ×100)

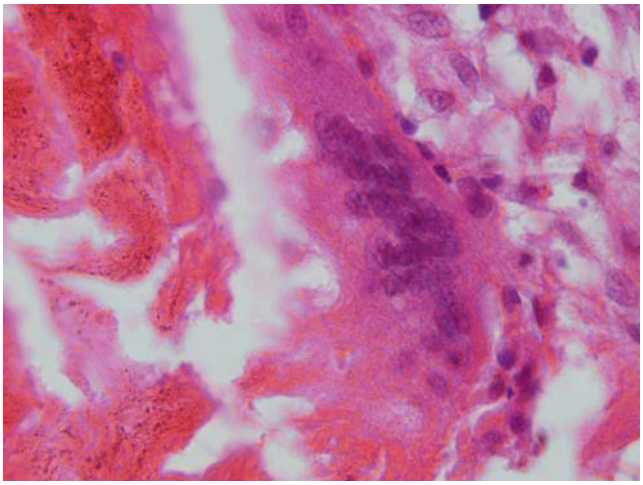

Fig. 4 Note the accumulation of macrophages or epithelioid cells and a few eosinophils around necrotic debris of the xenogenic graft (haematoxylin and eosin, ×1,000)

The main objective of the study was to determine whether an ostrich tendon graft could provide a grafting structure that enhanced the surgical repair of tendon defects. The results showed that the transplanted tendon was rejected and showed signs of degeneration in the transplanted area. The gross and microscopic observations almost 3 months after surgical transplantation indicated that all of the seven tendon xenografts were rejected by the recipient rabbits. There was microscopic evidence of significant tissue reaction in the rabbits with grafts. A vigorous inflammatory response was observed toward the xenogenic ostrich tendon, the degree of increased cellularity and the presence of extensive polymorphonuclear and monocyte cell infiltrates indicate that ostrich tendon is highly antigenic. This finding is supported by several studies that have shown inflammatory infiltrates composed of lymphocytes, plasma cells, histiocytes and polymorphonuclear leukocytes and a response characteristic of a giant cell foreign body reaction (Allen et al. 1987; Tauro et al. 1991; Milthorpe 1994).

In our study, degeneration and resorption of the grafted material were observed in gross evaluation. Milthorpe (1994) showed that xenogenic graft materials were resorbed rapidly and replaced with amorphous scar tissues by 3 weeks post-transplantation. However, a study by Dehghani et al (2005) showed successful transplantation of bovine foetal tendon in a rabbit model.

It has been reported that the processes of fresh-freezing, freeze-drying or cryo-preserving allograft tissue significantly reduces the immunogenicity of the tissue by killing

fibroblasts within (Arnoczky et al. 1986). Although we stored the ostrich tendon at −20ºC before transplantation, we still observed vigorous inflammatory reactions and signs of graft rejection in the histopathological evaluation.

Conclusion

Overall, our results showed that ostrich tendon exhibits severe anti-genicity and elicits vigorous inflammatory reaction as such its use is not recommended as a xenogenic tendon graft for the replacement of tendons or ligaments.

Acknowledgements This research was financially supported by the Research Council of Shahrekord University, Shahrekord, Iran.

References

Allen PR, Amis AA, Jones MM, Heatley FW (1987) Evaluation of preserved bovine tendon xenografts: a histological, biomechanical and clinical study. Biomaterials 8:146–152. doi:10.1016/0142-9612(87) 90105-0

Arnoczky SP, Warren RF, Ashlock MA (1986) Replacement of the anterior cruciate ligament using a patellar tendon allograft: an experimental study. J Bone Joint Surg Am 68-A:376–385

Barfred T (1973) Achilles tendon rupture. Aetiology and pathogenesis of subcutaneous rupture assessed on the basis of the literature and rupture experiments on rats. Acta Orthop Scand Suppl 3:126

Bertone AL (1995) Tendon lacerations. Vet Clin North Am Equine Pract 2:293–314

Best TM, Glisson RR, Seaber AV, Garrett WE Jr (1989) The response of muscle–tendon units of varying architecture to cyclic passive stretching. Trans Orthop Res Soc 14:294

Buckwalter JA, Hunziker EB (1996) Orthopaedics. Healing of bones, cartilages, tendons, and ligaments: a new era. Lancet 348:2. doi:10.1016/S0140-6736(96) 98028-9

Clancy WG (1983) Treatment of knee joint instability secondary to rupture of the posterior cruciate ligament. Report of a new procedure. J Bone Joint Surg Am 65(3):310–322

Dehghani SN, Varzandian S (2007) Biomech Study Repair Tendon Gap Bovine Fetal Tendon Transpl Horse. J Anim Vet 6:1101–1106

Dehghani SN, Tadjalli M, Akbari A (2005) Transplantation of bovine foetal tendon in rabbit flexor tendon. Veterinarski Arh 75:153–158

Dong DY, Sheng CY (1988) Study of permanent artificial tendon. Artif Cells Blood Substit Biotechnol 16:927–944. doi:10.3109/10731198809117278

Dyce KM, Sack WO, Wensing CJG (1996) Textbook of veterinary anatomy. W.B. Saunders Company, Philadelphia

Frank CB (1996) Ligament healing: current knowledge and clinical applications. J Am Acad Orthop Surg 4:74–83

Frank CB, Hart DA, Shrive NG (1999) Molecular biology and biomechanics of normal and healing ligaments—a review. Osteoarthritis Cartilage 7:130–140. doi:10.1053/joca.1998.0168

Gordia VK, Grana WA (2001) A comparison of outcome at 2 to 6 years of acute and chronic anterior cruciate ligaments reconstruction using hamstring tendon graft. Arthroscopy 17:383–392. doi:10.1053/jars.2001.21493

Harner CD, Livesay GA, Kashiwaguchi S, Fujie H, Choi NY, Woo SLY (1995) Comparative study of the size and shape of human anterior and posterior cruciate ligaments. J Orthop Res 13:429–434. doi:10.1002/jor.1100130317

Karakurum G, Güleç A, Büyükbebeci O, Karadag E (2003) The ostrich: an excellent tendon source for the biomechanical studies. Gulhane Med J 45:180–181

Kuo YR, Kuo MH, Chou WC, Liu YT, Lutz BS, Jeng SF (2003) One-stage reconstruction of soft tissue and achilles tendon defects using a composite free anterolateral thigh flap with vascularized fascia lata: clinical experience and functional assessment. Ann Plast Surg 50:149. doi:10.1097/01.SAP.0000037270.95257.B9

Lin TW, Cardenas L, Soslowsky LJ (2004) Biomechanics of tendon injury and repair. J Biomech 37:865–877. doi:10.1016/j.jbiomech.2003.11.005

McMaster WC (1985) Bovine xenograft collateral ligament replacement in the dog. J Orthop Res 3:492–498. doi:10.1002/jor.1100030412

Milthorpe BK (1994) Xenografts for tendon and ligament repair. Biomaterials 15:745–752. doi:10.1016/0142-9612(94) 90027-2

Rodeo SA (1993) Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. J Bone Joint Surg Am 75 (12):1795–1803

Rogers GJ, Milthorpe BK, Schindhelm K, Howlett CR, Roe S (1995) Shielding of augmented tendon-tendon repair. Biomaterials 16:803–807. doi:10.1016/0142-9612(95) 99643-Z

Scotish National Blood Transfusion Service (SNBTS) Tissue Service (2005) Instructions for thawing and use of tendon products. SNBTS, Edinburgh

Strickland SM, MacGillivray JD, Warren RF (2003) Anterior cruciate ligament reconstruction with allograft tendons. Orthop Clin North Am 34:41–47. doi:10.1016/S0030-5898(02) 00060-3

Taniguchi Y, Tamaki T (2000) Reconstruction of the achilles tendon and overlying skin defect with a medical plantar flap and tensor fasciae latae graft. J Reconstr Microsurg 16:423–425

Tauro JC, Parsons JR, Ricci DJ, Alexander H (1991) Comparison of bovine collagen xenografts to autografts in the rabbit. Clin Orthop Relat Res 266:271–284