Introduction

Rotator cuff tears are a major cause of morbidity with an incidence of up to 35%. Symptoms range from minimal pain or functional deficit to significant pain and disability.(1) Following rotator cuff tears, muscle atrophy and fatty infiltration begin in the supraspinatus muscle limiting repair potential. The gross and microscopic appearance of rotator cuff fatty infiltration progression is well documented, however the cause remains unclear. Research into the pathogenesis of these changes examines tear size and duration of un-repaired tears. Little is reported on the role the nerve may contribute to the development of fatty infiltration.(2) Recently a process of supraspinalu neuropathy was described resulting from excessive traction on the supraspinacu nerve in massive retracted tears.(3) We hypothesize that changes in the innervation at the motor-end plate contributes to fatty infiltration.

Materials and Methods

The study complied with the guidelines published by the National Institutes of Health and was approved by the Laboratory Animal Care and Use Committee at Wright State University.

Four 6-month old New Zealand white rabbits were randomized to receive injuries on either the right or left shoulder. Rabbits were anesthetized with an intramuscular injection of ketamine, xylazine, and atropine and maintained on isoflurane. The shoulder was shaved, aseptically prepared, and a 3 cm longitudinal incision was made over the deltoid muscle. The deltoid was retracted and the supraspinatus tendon was transected at its insertion on the greater tuberosity. All attachments of the tendon to the surrounding tissues, including the infraspinatus were released, allowing the tendon to retract. The cut end of the tendon was tagged with 4-0 Nylon suture for identification at harvest. The wounds were irrigated and closed with interrupted, subcutaneous 3-0 vicryl sutures, and the skin was closed with a continuous 4-0 monocryl suture. The contralateral shoulder served as an unoperated control. All rabbits tolerated this procedure without any intraoperative complications. Buprenorphine (0.1 mg/kg SQ) was given twice daily for 5 days for post-operative analgesia. One case of self-inflicted wound dehiscence occurred which required a second wound closure.

Animals were housed in standard-sized cages (0.47 m²) and were free to move about the cage unrestricted. All animals returned to normal weight bearing status within 10 days of surgery. At three months all four rabbits were euthanized. The supraspinatus muscle and supraspinal nerve were completely dissected from the supraspinatus fossa.

The supraspinatus was sectioned into multiple slices for motor end plate and histologic analysis. Sections for end plate analysis were postfixed in 4% paraformaldehyde for 24 hours, cryoprotected in 15% sucrose overnight, and then frozen in liquid nitrogen. Muscles were sectioned (20 µm thickness) and acetylcholine receptors (AChRs) in motor endplates were labeled with rhodamine-conjugated α-bungarotoxin ( Molecular Probes). Axons and motor terminals were labeled with a mouse monoclonal antibody against the phosphorylated heavy fragment of neurofilament protein (SMI31, 1:40, Developmental Studies Hybridoma Bank, University of Iowa). Labeling of both primary monoclonal antibodies was visualized using a fluorescein-conjugated donkey anti-mouse secondary antibody (1:100, Jackson Immunoresearch Laboratories).

Z-axis stacks of images at sequential focal planes (0.5 µm separation) of neuromuscular junctions were obtained using a Fluoview FV 1000 confocal microscope (Olympus optical). The illustrated images are flat-plane in focus projections obtained from z-series images using Fluoview software. Endplates were categorized as partially denervated if over 15% of the postsynaptic region labeled by α-bungarotoxin was unoccupied by nerve terminal staining. Paired student T-test was used to analyze the data with α=0.05.

Sections from the musculotendinous junction for histologic analysis were fixed in 10% neutral buffered formalin. Specimens were stained with osmium tetroxide for fat analysis. Images were taken with an Olympus DP72 camera on a BX50 microscope and fat to muscle ratio calculated using NIH image J.

Results

Atrophy and fatty infiltration were present in all 4 test specimens and in none of the control specimens. Fibrous tissues filled the gap between the

Rabbit Supraspinatus Motor Endplates are unaffected by a Rotator Cuff Tear


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Figure 1. Confocal Microscopy analysis. Representative images of supraspinatus muscle three months following transection of the supraspinatus tendon. In the low power image (upper row), a nerve can be seen entering in the lower center of the photograph and branching to innervate five endplates (number 1-5). All five endplates have normal tight clustering of acetylcholine receptors (AChRs) which are nicely aligned with the nerve (see overlay). In the high power image (lower row) two endplates are present. Each endplate is innervated by a single axon which aligns well with the AChRs.

Discussion

Our results demonstrated that rotator cuff tears do not lead to loss of the motor endplate or innervation status of the supraspinatus muscle 3 months after injury in the New Zealand white adult rabbit. A limitation of this study is that the induction of the lesion was a surgical resection and not a naturally occurring tear. Thus, pre-existing degenerative changes which occur in humans were not included in the model. In addition, the anatomical configuration of the shoulder of the rabbit may not accurately reflect what is occurring in humans. For example, compression of the supraspinal nerve may contribute to muscle alterations in humans whereas it may not be a contributing factor in rabbit supraspinatus injuries.

Clinically muscle weakness is typically observed at 25-50% denervation. The 1% reduction in motor end plate innervations in this study is thus not a significant biological alteration.

Understanding the source of fatty infiltration is important to orthopedic surgery because its presence can limit the success of rotator cuff repair. These results clearly demonstrate that in the rabbit, fatty infiltration is not caused by supraspinatus muscle motor end plate denervation.

References

2. Rubino LJ, Sprott DC, Stills HF, Jr, Crosby LA. Fatty infiltration does not significantly different between control and experimental muscle. On average there was 3.2% denervation and 1.2% partial denervation in the experimental muscle (control was 2.1% and 1.4% respectively).

Poster No. 538 • ORS 2011 Annual Meeting